

European research collaboration

The British will not be pulling out of the molecular biology laboratory at Heidelberg, but Europe needs urgently to explore ways of collaboration in research other than international laboratories.

THE British, who have earned themselves the unenviable reputation of not being reconciled to the notion that Britain is a part of Europe, have been making heavy weather in the past few years over their continued membership of the European Molecular Biology Laboratory, the international laboratory at Heidelberg in West Germany which is, confusingly, an offshoot of the European Molecular Biology Organization but constitutionally separate therefrom. The difficulties are several. When the foundation of a collaborative laboratory was first canvassed more than a decade ago, largely on the grounds that there was a need for an institution that would enliven European molecular biology as CERN at Geneva had enlivened European high-energy physics, the British Medical Research Council was one of the chief sceptics. In the pragmatic British tradition, the council argued that the analogy with CERN could be misleading, for molecular biology does not depend on the construction of huge central facilities, and that the mere existence of a central laboratory, by attracting bright people, might serve in fact to weaken national efforts in this important field.

In the event, the council's misgivings were stilled by political considerations. Ten countries (including Switzerland and Israel) signed a treaty setting up the laboratory in May 1973, and the Medical Research Council has since been the channel through which the annual British contribution to the laboratory is paid. But in that role, the council seems never to have exorcised its original doubts. The council's latest formal inquiry into the value of the work done at Heidelberg (see *Nature* 19 January, p.203) is the third occasion in ten years on which the council has thought it appropriate solemnly to appraise the value of the laboratory's scientific programme, although on this occasion it was on formal instructions from the Advisory Board on the Research Councils that continued membership of the laboratory was reviewed.

British blowing hot and cold in this way has had a predictably undermining effect at Heidelberg and among other members of the laboratory. It seems to have been widely understood that if the British chose to withdraw from the laboratory, others would follow. It has been especially disconcerting for the new director of the laboratory, Dr Lennart Philipson, that one of his chief sources of financial support (the British contribution to the laboratory is now nearly 17 per cent of the total) should be put in hazard within a few months of his appointment. Understandably, if wrongly, there have been complaints that the British were content with continuing membership only so long as a British national (Sir John Kendrew) was the director of the laboratory, and that they have seized on his departure (half-way through 1982) as the occasion for pulling out.

Moratorium

These are some of the reasons why the report of the committee of inquiry, now unexpectedly published, is so warmly to be welcomed. Its recommendation, that there should be a once and for all decision (to belong or to pull out) and then a moratorium on further introspection about membership of the laboratory, has been accepted by the research council. It is unthinkable that the Advisory Board on the Research Councils will not follow suit when it considers the matter this week.

So has the time come when people in Heidelberg can relax? Not quite. The latest British inquiry has been chiefly concerned with

the laboratory's record and with its programme for the years ahead. Its report acknowledges that the laboratory has provided distinctive facilities at its two outstations (neutron diffraction at Grenoble, synchrotron radiation at Hamburg), that some of the groups at the Heidelberg laboratory are working in fields which are comparatively neglected in Britain, that many of these projects offer the chance that collaboration will yield an effort greater than the sum of its parts and that there are, indeed, opportunities for creating central pools of expertise unlikely to be replicated in the laboratories of member states. The committee of inquiry also considered the relatively high cost of the Heidelberg laboratory and offered its advice that the extra price is worth paying for the sake of the benefits of collaboration. But the inquiry has not taken up the more important question for the future of European collaboration, that of the utility of the Heidelberg as a model for collaboration in other fields.

Scepticism

The truth is that some of the original scepticism about the need for a central laboratory can still be justified. Molecular biology is not, after all, high-energy physics and the case for the Heidelberg laboratory differs in important ways from that for CERN. That, at least, is the present position, although the founders of CERN were diligent advocates of the view that central facilities at Geneva should not be regarded as a substitute for national accelerators but, rather, as a spur to member states to build big facilities of their own. That doctrine of calculated extravagance has now mercifully been dropped and CERN's justification, for the time being, is that it has, or is building machines that no member state could afford. Its worry is (or should be) that even collaboration on a European scale may be insufficient to meet the cost of the next generation of particle accelerators and that the building of large accelerators may not even be the best way of stimulating high-energy physics (see *Nature* 1 December, p.421).

The case for the Heidelberg laboratory, originally cast in similar language, is now recognized to be intrinsically different. Although there are facilities not to be found elsewhere in Europe, their provision does not hang on the existence of a central laboratory, whose justification then becomes its capacity to attract distinctive people and problems. The unanswered question is whether the same benefits might be accomplished by other means, perhaps at less cost. If, for example, there were a grant-making body operating on a European scale in the support of molecular biology, the chances are that its funds would go on the support of projects not very different from those being assembled at Heidelberg. Similarly, if national research councils were more ready than they are at present to spend money on collaborative projects, and if their governments would let them follow such an enlightened policy, much the same might be accomplished. Collaboration along lines like these could, in the long run, be cheaper, more flexible and more productive. So the case for central laboratories on a European scale is largely psychological — they entail a visible commitment by member states which cannot easily be shuffled off.

There are several practical lessons to be drawn from this argument. On the Heidelberg laboratory, the British institutions which have found it impossible to bury their doubts about the wisdom of collaboration should do what the latest inquiry



recommends — pay up and do what they can to make a success of the venture. And because the venture implies a commitment not just by the Medical Research Council but by the British government, steps should be taken to see that the funds available for supporting academic research in the United Kingdom do not fluctuate with each variation of the international value of sterling or the relative economic growth of the partners in these collaborations. But none of this should be taken as a sign that European collaboration (of which there is too little) should always be organized around central laboratories. Indeed, there is the strongest case for deliberate experiments in the support of European research from central funds along the lines on which research councils function nationally. The European Molecular Biology Organization, which has won a high reputation in its administration of fellowships and training programmes, is ironically the most obvious vehicle for such an experiment. Is it outrageous to suggest that when the issue of the laboratory is settled, national research councils should bite harder on the bullet and set aside further modest funds for supporting less formal kinds of collaboration? □

Breaking all the rules

The US accuses the Soviet Union of violating arms agreements, but is breaking other rules.

THOSE who worry about the growing tension between the superpowers should remember that one of the earliest and most successful arms control agreements ever negotiated by the United States is already 167 years old. That agreement was not, of course, with the Soviet Union but with Great Britain. The Rush-Bagot agreement which, in 1817, demilitarized the Great Lakes is still regarded by historians as an outstanding instance of hostile powers entering into sensible arrangements to reduce the military friction between them. Yet for the first hundred years of its life, the treaty was the subject of heated accusations by both sides. It is conceded that its technical provisions have been violated countless times.

The US Congress would do well to keep in mind Rush-Bagot when it ponders the report it has now received from the Reagan Administration alleging that the Soviet Union is guilty of several violations of the fragile arms control regime now in place. Regrettable as they are, technical violations need not unmake a useful treaty unless the signatories choose to let them. That is why it is unfortunate that at least some members of the Reagan Administration have created the impression that they would like the still unpublished report to destroy what remains of American confidence in the trustworthiness of the Soviet Union, perhaps even to undermine the notion of arms control as such.

Why else should the administration have chosen to raise a public stink about the alleged violations before exhausting the opportunities that exist in the private Standing Consultative Commission (SCC) set up under Salt I and Salt II expressly to deal with problems of this kind? SCC has already proved itself a successful (but slow) mechanism. Between 1973 and 1977, discussions in SCC persuaded the United States that silos being constructed in the Soviet Union were indeed intended to house launch-control facilities and not new missiles. And in 1975, when radioactive material was vented into the atmosphere after a Soviet nuclear test in violation of the 1963 Limited Test Ban Treaty, private bilateral talks persuaded the Carter Administration that the technical violation had not undermined the value of that agreement.

The decision to complain in public would be more understandable if the administration had clear proof of the alleged violations, and if they were of serious military significance. Yet the United States concedes that only two of the seven violations have been confirmed to its own satisfaction and that neither of those — the alleged use of chemical and biological weapons in Afghanistan and a breach of the 1975 agreement on notification of military manoeuvres in Europe — is part of the bilateral nuclear agreements between the superpowers. The other allegations are more tentative. In some cases, such as the allegation that the

Soviets have tested above the 150 kiloton threshold set by the unratified Threshold Test Ban Treaty, the technical evidence is ambiguous. In others, like the encryption of telemetry during missile tests, the superpowers differ over the legal interpretation of the Salt II agreement.

Why, then, has the administration chosen this moment to raise public questions about the value of the treaties it has signed with the Soviet Union? One plausible though perhaps uncharitable explanation is that the administration is preparing public opinion for an abrogation of the Anti-Ballistic Missile (ABM) Treaty so that it can proceed more quickly with development of the "star wars" missile defence system proposed by President Reagan last March. There are signs enough for the congenitally suspicious that the President has decided to give the star wars plan his blessing. To the Soviet Union, for example, last week's first test by the United States of an antisatellite missile (see page 306) and the President's announcement (expected this week in the State of the Union speech) that the government has approved plans for a space station, are bound to look threatening.

A slightly more charitable explanation of the administration's behaviour is that its report on Soviet treaty violations is simply another unsuccessful attempt to paper over its own political troubles. It is an open secret that the administration is divided between those who favour new arms control measures and those who want to break out of the existing regime so that the United States can recover its dominant position in strategic weapons. Complaints in public on the basis of a still-secret document may have been a convenient compromise between the two factions. The effect, however, will be to make the Soviet Union even more reluctant than it already is to answer the serious charges against it. □

West German reform

The Bonn government seems bent on carrying reform to the universities, urgently in need of it.

LEGEND has it that the student prince was almost always penniless but invariably free — free to move from one university to another, to let his inclinations guide his pursuit of knowledge or even, if that seemed more appropriate, the pursuit of other goals. In the hard decades after the Second World War, the species has become almost extinct. Now, only vestiges of the old behaviour-pattern are to be seen. Italian universities have no effective mechanism except discomfort for turning away students whom they cannot hope to teach. In France, in spite of the troubles of 1968 which filled the streets of Paris with would-be student princes, the government seems cleverly to have managed to keep the worst excesses of the old system in check, helped no doubt by economic reality of the kind that touches students. Only in West Germany have the old ways been substantially unchanged, with the result that some universities (the University of Munich, for example) are unmanageably large while others, some of the newer creations in *Nordrhein-Westfalen*, are crying out for students. And the capacity of universities to manage their affairs efficiently has been further undermined by the basic law that distributes decision-making to statutory committees able, if so inclined, to exercise the rights but not the responsibilities of power.

Against this background, the new proposals for reform now in the mill at Bonn are much more radical (and welcome) than they may seem at first sight. The idea that students should be guided to the places where they can be taught is not illiberal (as some will make out), it does make sense that students should not remain students indefinitely, it is high time that some power of decision should be returned to those who are blamed if things go wrong and it is especially welcome that there should be a scheme for keeping people in the academic system for long enough to tell whether they will be able to find permanent places for themselves. The dangers now lie not so much in Bonn but with the backwoodsmen, the politicians in the *Länder* governments who do not see the need for reform and with the would-be student-princes. It will be interesting to see how many are left. □

US nuclear power

Cancellations promise bleak year ahead

Washington

THE new year has brought a new wave of trouble for the US nuclear power industry. Five nuclear power plants now under construction have either been cancelled or have encountered licensing problems in just the last few weeks, chiefly as a result of rising costs and newly discovered safety violations. And a report from the Energy Information Administration (EIA)* has found that average construction time for a new reactor has reached 12 years — more than twice the time it took to build a reactor in the early 1970s. Three-quarters of the plants completed in the past decade or nearing completion now have cost more than twice the original estimate. One in ten of them have cost more than seven times the original estimate.

These escalating costs, together with the failure of electricity demand to keep up with the utilities' projections, have played a large part in the recent cancellations. Last week, the Public Service Company of Indiana announced that it was abandoning the Marble Hill plant on which it has already spent \$2,500 million. The River Bend plant No.2 in Louisiana was recently cancelled as well, and the two-reactor Midland plant in Michigan is facing an uncertain future, with its current estimated cost at more than ten times the original figure of \$300 million.

Safety violations have aggravated economic difficulties in some other recent cases. On 13 January, the Nuclear Regulatory Commission's safety and licensing board unconditionally denied an operating permit to the Byron plant in Illinois, saying it had "no confidence" in the quality assurance programme of key contractors. Although the board did not point to specific safety deficiencies, it said that the quality assurance was so poor that there was no way to know for sure. According to the Nuclear Regulatory Commission press office, the board, in its 400-page report, charged that Systems Control Corporation, which supplied safety-related equipment, had actually run a "fraudulent and ineffective" quality assurance programme and is being investigated by the Department of Justice.

Spokesmen for the utility said the problems were only in the documentation of the quality assurance efforts, not in the work itself. They also said that the utility was losing \$1 million a day as a result of the delay.

Safety troubles with Cincinnati Gas and Electric's Zimmer plant led to an unusual decision over the weekend at that troubled plant, which has stood half-finished since November 1982 when mounting quality

assurance failings prompted the Nuclear Regulatory Commission to order a halt in construction. The utility decided that rather than spend an additional \$1,000 to \$2,000 million to complete the nuclear plant, they would convert the station to a coal-burning facility. The utility had already spent \$1,700 million.

A spokesman for the Atomic Industrial

West German universities

End of old order in sight?

A RADICAL reform of West German education is in the air. Professorial powers will be increased and advance beyond the basic degree will be made more difficult if recommendations made by a commission appointed by the West German Minister of Education are accepted.

The commission's mandate was to examine the working of the 1976 University Law (*Hochschulrahmengesetz*) framed in reaction to the student unrest of the late 1960s. This shifted the balance of power from the professors (the *Ordinarien-herrschaft*) to a wider group. Decision-making committees of the universities now comprise representatives including students, administrators, scientists and non-scientists, as well as professors.

The commission finds, in agreement with the *Wissenschaftsrat* and the Conference of West German Rectors, that over-diligent application of the principle of group responsibility has led to the hardening of conflicting interests, inefficiency and a mushrooming bureaucracy.

The commission wants to see the professorial and faculty role strengthened at all levels. However, the number of faculties should be limited to fifteen, and the heads of faculties (*Decane*) represented in the senate. It does not, however, agree with the rectors that the professorial complement in the senate should be increased. At present, university rectors are elected by the professors in universities of six *Länder*. The commission favours this system as against appointment by the president.

Subject to restrictions on admissions to popular faculties (*numerus clausus*) such as medicine, the school-leaving *Abitur* remains a general passport to university education and student careers have traditionally been long even with rising student numbers. The report now recommends that length of study should be controlled and academic criteria strictly applied to courses beyond the basic level.

Many bureaucratic barriers have arisen in the application of private sources of finance in university research. All such sup-

Forum said the cancellations were all primarily due to the low growth in electricity demand. He said that "nuclear is a natural target" because of the large capacity of nuclear plants and their high initial capital costs.

The cancellations of half-completed nuclear plants are already sparking heated confrontations over who is to bear the construction costs — stockholders or ratepayers. The decisions in each case will ultimately be made by the state regulatory commissions which have different rules and have come down on different sides of the issue.

Stephen Budiansky

*"1983 Survey of Nuclear Power Plant Construction Costs", DOE/EIA-0439(83).

port now requires special permission and must be channelled through the university administration. The present government favours closer links between industry and the universities and will therefore agree with the recommendation that restrictions on this source of money be lifted and also that researchers be given freedom to appoint their own staffs.

The report also recommends the redesigning of the academic echelons. The present two highest levels of C3 and C4 will be amalgamated, perhaps to provide a larger pool of professional expertise. The lower C2 grade and general all-purpose university assistant would vanish, to be replaced by a class of scientific assistants who would be in the process of qualifying.

Those obtaining the *Habilitation*, the formal German professorial qualification, would be guaranteed a four-year post as an assistant, giving them time to receive the necessary invitation to a full professorship and at the same time maintaining a reserve pool of available talent.

The report has been a long time in gestation. It reflects the opinion now widely held among West German academics that the reforms of the 1970s, which diffused power widely among statutory university committees and which further strengthened the legal rights of students and junior members of teaching staffs, failed to tackle the outstanding problems of excessive demand for places in some universities and the difficulty of placing young people in any but the most demeaning academically related posts.

The problems encountered in some *Länder* of making full use of newly built universities have helped to win approval for the new proposals in the *Wissenschaftsrat*, the constitutionally unique body representing the federal and *Länder* governments which has been for the past thirty years the most powerful arbiter of policy on higher education.

The report will be the basis for discussion from which a new university law will emerge.

Sarah Tooze

Antisatellite weapons

First US test of airborne system

Washington

MORE than a year of lobbying by prominent scientists failed to prevent the US Air Force from conducting its first test-firing last Saturday of a new antisatellite weapon (ASAT) that can be launched from underneath the wing of an ordinary F15 fighter aircraft. The test, in which the missile was fired into space but not at a target, was immediately denounced as the prelude to a dangerous new phase in the arms race with the Soviet Union.

Development of the new weapon has been justified by the Department of Defense on the grounds that the Soviet Union is already believed to possess an operational antisatellite system. But critics complain that the American air-launched system is far more sophisticated than the Soviet system and, because of its small size, will make verification of a treaty banning such weapons virtually impossible.

The Soviet Union is believed to have conducted some 20 tests of its own main ASAT system, in which an explosive interceptor is launched from the ground by a variant of the SS-9 Scarp rocket. Last March, the Pentagon claimed that the Soviet Union would be able to launch the prototype of a space-based laser weapon some time after the mid-1980s and that an operational system capable of destroying satellites within a few thousand kilometres could be completed by the end of the century.

Unlike the existing Soviet system, the American weapon tested last week is launched in flight by an F15 aircraft and consists of a miniature non-explosive homing vehicle carried into space by a two-stage rocket. The vehicle would locate its target with infrared sensors and destroy it by collision. Congress has told the Air Force not to test the new weapon against target satellites until President Reagan provides a report on the administration's efforts to curb an arms race in space.

The administration is not expected to face any substantial difficulty in persuading Congress that the tests should go ahead, however. In recent months the Pentagon has been stressing the rapid strides the Soviet Union is believed to be making in space weapons, and a report last week in *Aviation Week and Space Technology* says the Central Intelligence Agency (CIA) believes the Soviet Union is developing a space-based defence system that could destroy American ballistic missiles launched against the Soviet Union.

At present there are no international treaties forbidding the deployment of ASAT weapons. The 1963 Limited Test Ban Treaty and the 1967 Outer Space Treaty prohibit the placing of nuclear weapons or other weapons of mass destruction in space, and the provisions of the 1982 Salt I treaty prohibit interference with reconnaissance satellites. Negotiations on a treat-

ty to ban ASATs were, however, broken off by the Carter Administration in 1979 after the despatch of Soviet troops to Afghanistan.

Many prominent American scientists have urged the Reagan Administration to take up recent Soviet offers to resume the stalled negotiations. Condemning last

week's ASAT tests, the group argues that the deployment of small and sophisticated ASATs would make the negotiation of a verifiable treaty almost impossible. Signatories of a statement criticizing the test include Jerome Wiesner, a former presidential science adviser; Hans Bethe, a physicist from Cornell University; Franklin Long, former associate director of the Arms Control and Disarmament Agency; and Herbert Scoville, former deputy director of CIA.

Peter David

Human rights

Philippines under scrutiny

Washington

ALLEGATIONS of gross violations of human rights in the Philippines have been made public by a delegation sent there by the American Association for the Advancement of Science (AAAS), the Institute of Medicine and four other scientific societies and human rights groups. Among the delegation's complaints are allegations of torture, "disappearances" of political detainees on the recent Argentinian model and the intimidation of health workers in rural areas.

The delegation was led by Dr Jonathan Vine, a private physician from Boston, and included Dr Robert Lawrence, director of primary care at the Harvard Medical School, and Eric Stover, staff officer of the AAAS committee on scientific freedom and responsibility. During their three-week stay in the Philippines, members of the delegation met government officials, Catholic clergy, health workers, US embassy officials, some of those in detention and also former detainees. The group visited seven detention centres and a variety of rural health facilities.

The rural health workers especially at risk are predominantly those employed on projects set up by the Catholic church to bring health care to remote areas not served by government facilities. According to the delegation, these workers are under constant scrutiny by the military, chiefly because of the suspicion that they are providing assistance for the guerilla force called the New People's Army. Many lay workers for these rural health projects have allegedly been detained and several private physicians have been arrested and held under "Presidential Commitment Orders" authorized by a decree suspending habeas corpus.

One of the incidents described in the delegation's report is that of the assassination in 1982 of a private physician, Dr Bobby de la Paz, who had operated a clinic on the remote Sumar Island. The local army command at first accused the guerillas but, in response to demands for a more thorough investigation, charged an enlisted man with the crime. The family of Dr de la Paz now claims, however, that the man thus identified does not match witnesses' descriptions of the assassin.

The delegation seems also to have been able to document several apparent cases of the torture of prisoners held for subversive activities. Its report includes a gruesome X-ray of the skull of a 37-year-old farmer who had a 4-inch nail driven into his skull while in detention. The police are said to have told the hospital to which the man was eventually taken that he had attempted suicide.

Clergy and others interviewed are reported to have said that there had been no substantial improvement of human rights in the Philippines since the lifting of martial law in 1981. The delegation notes, however, as an encouraging sign, the recent release of three scientists who had been in detention.

Stephen Budiansky

NIH curbs drive out researcher

Washington

A FEDERAL rule that forbids researchers at the National Institutes of Health (NIH) from conducting experiments on *in vitro* fertilization with human beings has driven out the chief of pregnancy research there. Dr Gary Hodgen, a leading researcher on human reproduction who has been at NIH for 15 years, announced last week that he will be leaving at the end of June to join a research group at the Institute for Reproductive Medicine at Norfolk, Virginia. The institute was among the pioneers of *in vitro* fertilization in the United States.

Under an administrative rule adopted during President Gerald Ford's administration, federal researchers are not allowed to use the procedures of *in vitro* fertilization or to carry out experiments in which human embryos are used. NIH regulations also forbid research with human fetuses except when the objectives include assuring the survival of the fetus or the avoidance of congenital defects.

The Institute for Reproductive Medicine at Norfolk is run by Drs Howard and Georgeanna Jones, a husband and wife team who have carried out some 50 successful *in vitro* fertilizations since 1982. Hodgen is to become the institute's scientific director.

Stephen Budiansky

UK nuclear power

Next reactor still not decided

THE public inquiry into the application from the Central Electricity Generating Board (CEGB) to build Britain's first pressurized water reactor (PWR) in Suffolk has now entered its second year, with no prospect of finishing before the autumn. It might easily take a further six to nine months for the Inspector, Sir Frank Layfield, to complete his report and submit it with recommendations to the Secretary of State for the Environment, who will take the final decision on whether the board should be allowed to go ahead.

The inquiry is being held in a former malting-house now converted into a fine concert hall, in the tiny village of Snape in Suffolk. The planned site for the PWR is just a few miles away at Sizewell, near an existing gas-cooled nuclear power station. The inquiry hall could hold 800 spectators, but since early last year it has rarely held more than a handful of people. All the activity takes place on a raised platform where Sir Frank sits, surrounded by crates of documents and under the continual glow of spotlights, attended by two assessors and an assortment of lawyers.

CEGB has a similar posse of lawyers on the platform ready to jump up and introduce procedural points, while the objectors, running their case on a limited budget, have a smaller contingent but manage to keep the words flowing.

A great many words are involved. Every one is taken down in shorthand, and the inquiry's secretariat now estimates that, including supporting documents, it distributes 200,000 sheets of paper a week. The

town, Ipswich. More often than not, however, the bus is empty. The one pub in the vicinity appears to be enjoying a good trade from board employees and objectors alike, although one person who is not to be seen in *The Plough and Sail* is Sir Frank Layfield: protocol demands that he is not seen dining, and presumably drinking, with either party.

The first three months of the inquiry were taken up by the electricity utility reading out its evidence, and were followed by nine months of detailed examination of the economic justification for the board's case. The objectors claim to have significantly weakened it: the board itself admits to having refined some of its assumptions but insists the case holds. Now, attention has turned to safety aspects.

Friends of the Earth, the British environmental group, has hired a barrister to make its case that the board's design is not acceptably safe, and reckons to have spent £100,000 on the inquiry so far. The group will retire from the inquiry in a few weeks' time, to be replaced by the Stop Sizewell B Association, which will concentrate on attacking the board's operational safety

claims. In particular, the association will be pressing that the design should include electromagnetic filters in the reactor's coolant circuit at a cost £5 million. The filters will, it is claimed, reduce radiation doses to operators by 50 per cent.

This week, Friends of the Earth intends to raise the temperature of the proceedings by introducing a document produced by the UK Atomic Energy Authority but not publicly available in Britain. The document provisionally endorses a controversial study by the United States Oak Ridge National Laboratories which found actual rates of mishaps in reactors significantly in excess of predicted rates. Friends of the Earth says the document became available only because of an application in the United States under the Freedom of Information Act, and that the study of the frequency of mishaps at British reactors had been carried out under contract for a US customer.

If CEGB's application is granted, the result will be the introduction of a new type of reactor technology into Britain. The board's previous experience with building power stations is not encouraging, but it says that the PWR could be built in a record 19 months once it gets clearance. However, plans are already two years behind schedule and costs are rising. **Tim Beardsley**

Indian universities

Can academic politics be banned?

New Delhi

INDIA'S University Grants Commission (UGC) has called on the ruling political parties in the central and state governments to "set an example" by refraining from interfering in the internal affairs of universities. At the same time, the commission urged academics and students to avoid bringing politics into university matters. Universities cannot be run effectively, Mrs Madhuri R. Shah, head of UGC, observed, while the present "free for all" is considered to be an expression of "democracy" in the universities.

Among those spheres of academic life which UGC considers should be free from "politics" are appointments, recruitment, admissions and some administrative and financial matters.

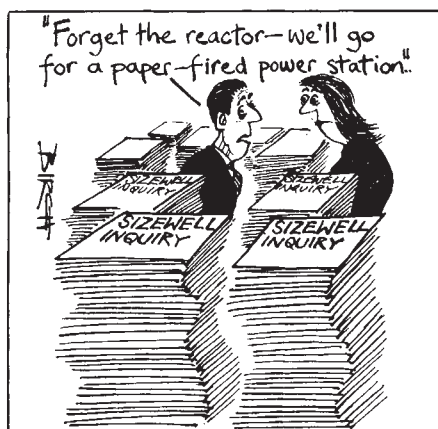
All these spheres have, of late, become imbued with politics. Thus a recent dispute over the appointment of a new vice-chancellor at the University of Calcutta developed into a major confrontation (complete with students' strike) between the governor of West Bengal, Mr A.P. Sharma (who is *ex officio* chancellor of the university) and the Left Front state government. And a student campaign in Bangalore ostensibly demanding universal access to "scientific and democratic" higher education is closely linked with the conflict between the Janata state government and the Central Congress (I) government, and the opposition in southern India

to Mrs Gandhi's hints that Hindi should replace English as India's "link language". In Assam, the All-Assam Students Union is allegedly involved in plans to bring down the state legislature.

UGC therefore suggests a formal separation of political and university life. Central and state ministers, it urges, should not seek appointment to university bodies, and university lecturers and employees who are elected to central or state legislative assemblies should be granted unpaid leave during their term of office.

University vice-chancellors should be given the "full trust" of government officials instead of, as often happens at present, the latter giving their overt or covert support to groups opposed to the university authorities. In the seven central universities (which are supported by the Delhi government), vice-chancellors should have direct access to the "highest quarters" of government, in recognition of their status as national institutions.

UGC does not spell out how these objectives might be attained, but simply appeals to the "maturity" of the political parties, calling on them to unite to save the universities "which have a key role in building the country's future". In the present atmosphere of tension between the central and state governments and the current militancy of Indian students, however, such exhortations seem somewhat utopian. **Vera Rich**



proportion of these that are ever read is a different matter.

Local residents have mixed feelings about the effects of the inquiry on Snape itself, but there can be no doubt that money from CEGB has largely financed the conversion of the concert hall complex into a potentially profitable leisure centre, and that jobs will be created if the new reactor is built. Under the terms of the inquiry, the board pays for everything, from the inspector's salary down to the bus that every day runs to and from the nearest

Halley's comet

Europe's hopes for second probe

GIOTTO, the European Space Agency's first interplanetary satellite, designed to intercept Halley's comet, should be backed up by a second version which could be built at relatively little extra cost, some Europeans claim. Spare parts and a spare framework are already available, and could be assembled to make a Giotto II.

British Aerospace, prime contractors for Giotto, was not prepared last week to quote a price for such a project but, according to some estimates, a second Giotto, including launch, would cost only half as much as the current mission: another £50 million on top of the present £100 million.

The advantages of a second Giotto would be as a safeguard against the failure of the Ariane launcher, two of whose last six launches have failed. But if that launch were successful, a second Giotto could provide a matching experiment to send to another comet. The other missions to Halley, the Soviet Union's Vega craft and Japan's Planet-A and MST-5, are already double missions.

"This is a hot potato in ESA (European Space Agency) at the moment", said a Giotto specialist last week. It would put the £80-million-a-year ESA scientific programme back only a few months, he claimed, saying that it is "up to the scientific community" to decide whether it wanted to do it.

One planetologist in favour of a second spacecraft is Tony McDonnell of the University of Canterbury. He has already proposed a mission called Comex, which would be a modified Giotto II designed to intercept a number of comets by using direct orbital injection from Ariane and by carrying a liquid fuel motor aboard. Comets differ greatly from one another, McDonnell points out, some bursting, others splitting with a variety of tails and emission characteristics, and there is a danger that the Halley data will give too selective a view of cometary structure and chemistry.

ESA planners, however, take the view that any modification of the tightly-designed Giotto mission would entail its total redesign and thus destroy the advantages of using Giotto spare parts. Giotto uses a solid fuel booster which can only be fired once to reach its interception orbit

with Halley, and so a Giotto II could be used effectively only for another single comet.

There may, however, be other arguments for a Giotto II. Giotto will go far closer to the comet nucleus than any other mission. (The closest approach will be less than 1,000 km, compared with upwards of 10,000 km for the Soviet and Japanese probes and for the American ISEE satellite recently diverted to comet Jacobini-Zimmer.) So, says Giotto's project scientist Ruediger Reinhard, Giotto has only a small chance of surviving the encounter.

Moreover, it is now clearly admitted, impacts with cometary dust in at least the last "tens of seconds" of the 30-minute passage of Giotto through the cometary coma to the nucleus are likely to throw out the close (1°) alignment of telemetry with the Earth, so that the last portion of data is in danger of being lost. And this assumes the dust cloud to be uniform; if Giotto were unfortunate enough to meet a jet of emission (such as are observed), misalignment would occur earlier.

There will be attempts to steer Giotto between any jets observed from Earth by taking the best straight-line path. But it is agreed that the whole exercise is risky. "We will know the risk sometime between 1830 hrs on 13 March 1986 and 0330 hrs on the next day", when the encounter takes place, said a British Aerospace spokesman.

● A rumour is circulating at the British Aerospace Dynamics Group near Bristol, where Giotto is being assembled, that Mrs Margaret Thatcher the British Prime Minister has enquired of an official whether Halley would be destroyed if Giotto targetting were so good that the satellite hit the nucleus (which is believed to be about 3 km in radius). The political consequences of such a conflagration would clearly be incalculable. But whether or not the question was actually put, British Aerospace did tackle the calculation. Little damage would be done, it seems, even though the impact would take place at 68 km per second. This velocity gives Giotto kinetic energy equivalent to about 250 kilotons of TNT, but the latent heat of melting of the cometary snowball would be some 100 million times greater.

Robert Walgate

European pollution

West Germany takes the lead

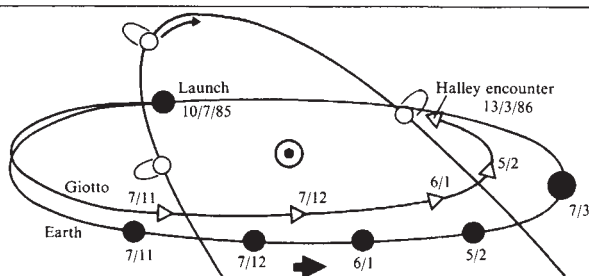
WEST Germany is well on its way to leading the European Economic Community (EEC) in environmental legislation. The impetus is the growing anxiety about the condition of West German forests (see figure opposite, and *Nature* 307, 97; 1983). A government committee, *Der Rat von Sachverständigen für Umweltfragen*, has now stressed that the phenomenon "can no longer be explained on the basis of traditional forest experience", but that acid air pollution must be an important contributory cause. But there are now also fears that air pollution is increasing mortality among the vulnerable trees — the old and the very young. Mortality among the young may be especially severe in West Germany, which now has the lowest success rate for new trees in the world.

The response to the anxiety has been impressive. In a debate in the Bundestag, the Federal Minister of the Interior, Dr Friedrich Zimmermann outlined a series of measures being taken in West Germany. The federal government is spending DM56 million (£14 million) a year on research related to forest death (compared with an estimated £0.5 million in the United Kingdom. Individual *Länder* are also reacting — Baden-Württemberg, which sent a delegation to Japan to investigate power-plant desulphurizing techniques, now has three pilot plants built at a total cost of DM 60 million (£15 million) without federal or EEC support.

West Germany emits 3.5 million tonnes of sulphur dioxide a year into the atmosphere, 43 per cent of it from power stations. Regulations introduced last year set the emission levels for new power plants at 1,800 mg m⁻³ and twice that for old plant. The utilities claim the new regulations will cost them DM10,000–15,000 million (£2,500–3,740 million); 20–30 per cent of the cost of new plant is already attributed to measures designed to protect the environment.

The West German environment council is nevertheless pressing for the emission levels to be further reduced, perhaps closer to the Japanese limit of 100–500 mg m⁻³. Even so, new regulations would reduce total annual emission of sulphur dioxide to 0.55 million tonnes a year within 10 years. (The opening by *Rheinischen Braunkohlenwerke AG* of what will be the largest brown coal mine in the world, with reserves of 2,500 million tonnes to be exploited over the next 50 years, may make that harder to achieve.)

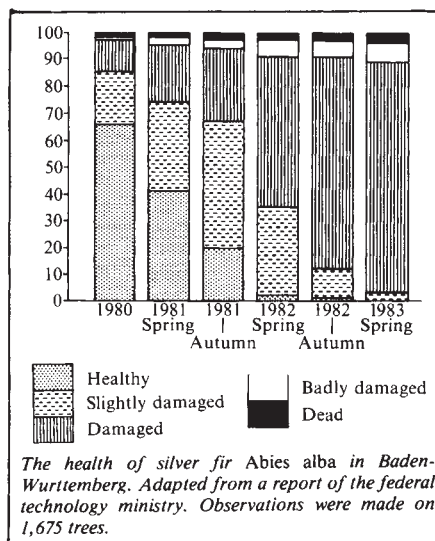
Nitrogen oxides from vehicle exhausts are another target for current concern. One controversial estimate is that the introduction of a speed limit, of 80 kilometres per hour in general and 100 kilometres per hour for autobahns, would reduce emis-



The orbits of Halley, Earth and Giotto, assuming a launch on 10 July 1985.

sions of nitrogen oxides by 13 per cent. But the German speed lobby has more than a little in common with the gun lobby in the United States.

In the European Communities as a whole, forest covers one-fifth of the total land area (some 35 million hectares) and provides employment for 1.4 million people. Nevertheless, the EEC is an importer of wood, bringing in 60 per cent of its needs in 1980.



Damage to forests such as has been found in West Germany is widespread throughout the Community and two proposals for directives are now being considered in Brussels. One would limit emissions from large installations to 250 mg m⁻³ by 1995 and reduce total emissions by 60 per cent for sulphur dioxide, 40 per cent for nitrogen oxides and 40 per cent for dust. The height of flue stacks would not be allowed to exceed 200 metres except under exceptional circumstances, limiting long range transport of pollutants. A second proposal on nitrogen oxides would forbid air concentrations above 200 mg m⁻³ for more than 2 per cent of the time. Both proposals include requirements for strengthening the monitoring system and supplying environmental information to Brussels.

Not all the economic considerations are negative. A study commissioned by the EEC estimates that air pollution from sulphur dioxide and nitrogen oxides costs the Community US \$1,400–4,200 million a year without taking account of damage to historic monuments and setting the cost of damage to public health at only \$20 million. The cost of control measures for large installations is estimated at \$370 million per annum for 25 years.

Sarah Tooze

● THE environment committee of the European parliament has now entered the fray on acid rain. The committee last week tabled a heavily critical report based on public hearings and written evidence, in which it claims that damage caused by acid dust and rain goes beyond even the 3–5 per cent of European community gross

national product estimated by the Organization for Economic Cooperation and Development. The report calls for heavy investment in smoke-scrubbing equipment, rapid legislation to limit emissions and for more research.

On Monday, the chairman of the committee, Scottish Labour MEP Kenneth Collins, said he hoped to put heavy pressure on the European Commission, and the decision-making council of ministers, to take action. There is "widespread agreement" in the council, said Collins, but one member state — the United Kingdom — was resisting all measures in the absence of incontrovertible evidence both of acid rain damage and of the origin of the acidity.

But evidence of this kind will never be watertight, says Collins. Moreover, the United Kingdom wished to wait at least until the findings of the Royal Society study, performed in conjunction with CEEB and the National Coal Board, and this might take five years.

Collins is hoping for some pressure on the United Kingdom from the French, during the present six months' French

presidency of the European Council. France is only just beginning to feel the effects of acid rain, largely in the Vosges in the east of the country, but it is more willing to act than the United Kingdom, Collins believes.

Meanwhile further pressure may come from the environment committee of the UK House of Commons, which will begin an investigation of acid rain within the next few weeks. Members of the committee will be visiting European countries including West Germany and Scandinavia.

And acid rain is now beginning to cause fears outside Europe and the United States, according to the International Union for Conservation of Nature and Natural Resources (IUCN). In response to a worldwide request for information, IUCN has learned of fears in South Africa for the Eastern Transvaal Highveld, which lie downwind of coal-oil conversion plants and coal-fired power stations centred on Witwatersrand and Johannesburg, and acidification down to a pH of 3.7–4.7 in San Paulo State, Brazil. IUCN has established a task force to study the worldwide effects of acidification. **Robert Walgate**

Afforestation

UK woodland policy disputed

A REVIEW of forestry policy in the United Kingdom by the British Association of Nature Conservationists has now concluded that the decline of ancient semi-natural woodland in Britain is being encouraged by a tax loophole. The review, entitled *The Future of Forestry*, endorses a Treasury study of 1971 which found that new afforestation is not justified in the national interest either strategically or economically. That report was overtaken by political events and by a government decision in favour of afforestation.

Now, according to the conservationists' report, private foresters are able, by changing ownership when a plantation becomes profitable, to switch between tax schedules so that initial costs are set against other income while productive stands are taxed only at their land value. The Inland Revenue accepts that these provisions are being exploited by syndicates of high-rate taxpayers but appears unable to remedy the situation. The annual loss through this "unscrutinized subsidy" may be up to £25 million.

Plantation with conifers, which give better commercial returns, often has damaging effects on wildlife, says the association. This is true especially where a high proportion of the land area is covered, as in parts of southern Scotland. The association recommends that the Forestry Commission, which is now disposing of many of its plantations in accordance with government policy, should cease further coniferization and that it should recommend private foresters to do likewise.

The report also bemoans the conser-

vation grant system which allows the Department of the Environment, through the Nature Conservancy Council, to buy woodland thought worthy of conservation from the Forestry Commission, which simply returns the money to the Treasury.

The Forestry Commission has yet to consider its reply to the charges made against it, but officials say that the decline of ancient woodland will not continue under present policies and that the commission already encourages plantation of broad-leaved woodland.

Private foresters are represented by the Royal Society of Foresters in England and Wales. Mr Esmond Harris, director of the society, objects to foresters' taxation arrangements being described as "manipulation". "They are," he says, "an incentive comparable with the government's Small Business Investment Scheme." And he argues that it is "sensible" to expand the home base.

Mr Harris also challenges the view that afforestation with conifers is damaging to wildlife interests. He claims the re-establishment of goshawks in Britain and the increasing numbers of golden eagles as proof of this, citing the support of scientists at the Institute of Terrestrial Ecology for this view.

According to Dr Ian Newton of the Institute of Terrestrial Ecology, the main complaint against coniferization is when it is done in blanket fashion. He also points out that, as the golden eagle needs open ground in which to hunt, its recovery can hardly be claimed as a success for forestry policies. **Tim Beardsley**

UK research

Review of research by numbers

THE Prime Minister, Mrs Margaret Thatcher, announced in the House of Commons last week the publication of the first of a series of annual reviews of government-supported research and development. The need for such a review was argued in 1981 by the House of Lords Select Committee on Science and Technology, which doubted the ability of the Treasury adequately to judge individual departments' research programmes against their responsibilities.

The science and technology secretariat at the Cabinet Office has carried out the first review which enables some trends to be discerned. As a public document, the review is ostentatiously laconic, replete with figures showing the sums of money spent by various government departments (which are listed consecutively) but entirely free from value judgements.

Nevertheless, the review touches base with most of the issues now causing anxiety within the British system of research and higher education. It refers to the dual-support system (but does not say whether it has broken down), mentions the problems encountered by the research councils in paying overseas subscriptions (but recommending no specific remedies), and so on.

It is not immediately apparent whether this is the kind of document for which the House of Lords was asking two years ago, although the political problems that would arise if the Cabinet Office were seen publicly to have a view on how government departments conduct their affairs are readily appreciated.

In one respect, however, the review does break new ground in Britain by adopting as the basis for its calculations a set of definitions of basic research, applied research and development which were first promulgated by the Organization for Economic Cooperation and Development (OECD) in its "Frascati Manual".

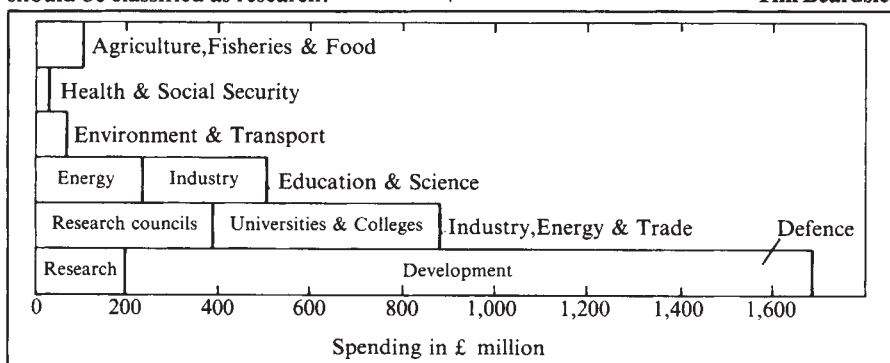
Evidently the Cabinet Office intends to apply these definitions to the classification of British research and development, raising in the process questions such as whether the collection of data as part of some routine operation, or the application of known knowledge to concrete tasks, should be classified as research.

On the figures now published, in which the most recent records are for 1981-82, defence spending shows the biggest increase between 1973/74 and 1981/82 while the proportion of all government-supported research and development by the Trade and Industry and Energy departments fell from 21.5 to 15.4 per cent over the same period. Expenditure by the Department of Education and Science shows a modest increase, from 24.6 to 27.4 per cent of the total.

The decrease in the figures for the Department of Trade and Industry is attributed to the running down of the programmes of support for the RB-211 aeroengine and Concorde civil aircraft. The large figure for defence includes the cost of development of specific items of equipment. □

● The increase in the share of the Department of Education and Science is a surprise. At a meeting in London last week, Dr John Burnett, Principal of the University of Edinburgh, argued that in the university sector, forward commitments on research equipment now exceed the ability of the system to pay for them. But the steep drop of support from the University Grants Committee in 1981 was preceded by a period of declining support for equipment and consumables, according to Sir David Philips, chairman of the Advisory Board for the Research Councils. And Professor John Kingman, chairman of the Science and Engineering Research Council, said that the diminished ability of his council to accede to all first-rate project grant applications was in part a consequence of the increasing sophistication of equipment but that government support for particular initiatives, such as that in information technology, had come out of the science budget. Not every body agrees that academic research is being hurt however. Drs Ben Martin and John Irvine of the Science Policy Research Unit at the University of Sussex argued that the evidence for a decline is largely anecdotal, and advocated a new approach to monitoring research output: the peer-review system, they said, might not be an adequate way of making decisions about closures.

Tim Beardsley



Information technology

Euoplan in doldrums

ESPRIT, Europe's £1,000-million three-year plan for joint pre-competitive research in information technology, hung in the balance earlier this week as foreign ministers clashed in Brussels over the future finances of the European Communities. Esprit was to begin on 1 January, but the wrangles over British and German contributions to the EEC budget, and over Europe's enormous agricultural surpluses (bought in by Brussels at supported prices) have brought the project to a halt. On Monday the EEC commissioner for research, Viscount Etienne Davignon, was berating ministers for ignoring Esprit and he claimed, in effect, that the economic future of Europe might depend on it.

Davignon's future certainly does, for Esprit is the *leitmotif* of his efforts to use the Brussels machinery to establish a truly European high-technology industry and infrastructure. But is his moment passing?

Esprit is already formally approved, but will have no money, probably until Britain and West Germany agree to disentangle the issue from the wider problems of the community. British officials have been hopeful, West German officials non-committal, about the prospects of this happening. Meanwhile both countries have been forging ahead with their own national plans. In both countries, companies are clearly divided about allegiance to Esprit or a national programme.

And beyond that, three European companies — Britain's ICL, France's Bull and Germany's Siemens — have already established a joint research centre (in Munich) said to be going so well that other companies are beginning to see the advantages of purely private cooperation.

In Britain, the "Alvey directorate", running a five-year £500 million programme in information technology, is getting wind of a new industrial view: that Brussels should concern itself more with the problem of setting standards (communications and design protocols and so on) than with research itself. This is but a minor part of the Esprit concept as it stands.

What seems to have happened is that the seductive possibility that one nation might win the information battle alone has taken hold. According to Mr Brian Oakley, director of the Alvey programme, Britain, with by far the fastest-growing population of microcomputers in Europe, is faced with "an incredible opportunity". This may be for the wrong reasons — people are playing "Pac-man" and "Space Invaders" rather than writing programs. But Oakley believes there is no smoke without fire.

Oakley is also proud of his team in the directorate, most of them on secondment from industry and still paid by their original employers. This arrangement has

caused some companies to fear loss of confidentiality, but those have been overcome, Oakley says. Even so, after Oakley's many years in government, he can say "I have never been in such a position where I can find out so rapidly exactly what companies think". With his £500 million, this gives Oakley a position of power and pulse-taking that Davignon or his staff would clearly like to exercise on the European scale.

There is some evidence that West Germany may follow the British lead. A plan for information technology is said to be in gestation — it will probably go to the German parliament in March, after the 28 February deadline Davignon seeks for agreement on Esprit. The upshot, that the two main complainants over the EEC budget may have made their own arrangements by them, does not augur well for Esprit.

Robert Walgate

East German chemicals

Back to coal

EAST Germany is planning a return to coal and, in particular, to domestically available lignite as the basis of its chemical industry, according to a report from the German Institute for Economic Research in West Berlin. Carbon chemistry has declined in importance in East Germany since 1975, with oil being used as the primary chemical feedstock.

Last November, at a meeting of the Central Committee of the United Socialist Party (SED), party leader Erich Honecker stressed that he attached the "greatest importance" to coal gasification and liquefaction. Current plans call for 80 per cent of the growth in lignite production to be allotted as a feedstock for the chemical industry. Expansion of coal tar and, above all, carbide production is a prime objective.

According to Mr Honecker, processing chemistry — including coal and lignite — ranks with biotechnology and microelectronics among the sciences which must "increasingly permeate" the East German economy. The target date for the large-scale liquefaction of lignite is, at the latest, 1992, with a planned annual processing of 10 million tonnes of lignite into fuel and liquid feedstocks. A team of 150 scientists at the carbo-chemical centre in Boehlen is said to be working on new sophisticated processes for lignite gasification and processing.

At present, however, the return to coal and acetylene seems to be based on a revival of old methods and technologies. This, according to the German Institute for Economic Research, involves a considerable loss of labour productivity and an increase in energy consumption, since in existing plants, nine to ten tonnes of raw lignite and a "very high input" of hydrogen are needed to replace one tonne of oil.

Vera Rich

US-Soviet space research

Moves for joint programme

MOSCOW radio's English-language service for North America has dropped several hints during the past few weeks suggesting a Soviet willingness to resume joint research projects in space. Such cooperation has, in fact, never entirely stopped. There is still some joint work on space medicine — including animal experiments on calcium loss due to weightlessness — and Soviet-American meetings of planetologists involved in Venus studies.

The recent hints suggest, however, that the Soviet side would like to see a resumption of plans for a joint manned mission. One commentator noted that there was at one time a plan for a link-up between the US space shuttle and a Soviet Salyut station. This, he claimed, was "unilaterally suspended" by the American administration, which has "increasingly been expanding military space projects at the expense of scientific research in space".

The same attitude of blaming the Americans for the cessation of these plans was adopted by Vladislav Dobrosel'skii, head of the external relations administration of the Soviet Academy of Sciences. He maintained that, in spite of highly-publicized official embargoes, the Washington government has quietly maintained a policy of preserving exchanges and contacts in areas where the Soviet Union is undoubtedly ahead. In the field of space medicine and biology, in which Soviet achievements are "obvious", the Soviet Union had however been guided by "humanitarian motives" in making their experience and knowhow available to the

Americans.

Ostensibly, Dobrosel'skii's call was for a restoration of "broad scientific ties" between the United States and the Soviet Union. His stress on the Soviet lead position in space, and the advantages which, even now, the Americans receive from it, suggests that renewed cooperation in space was the prime purpose of the broadcast.

What political ambience the Soviet Union would demand as a prerequisite for a major joint space experiment has not, so far, been spelled out. (So far the commentators have simply urged that such cooperation would be conducive to peace and detente.) Dobrosel'skii himself urged that cooperation should be "on a footing of equality, without any discrimination" and "free of contingency fluctuations" (presumably of political origin). It is possible, therefore, that the suggestions were intended as a warm-up to the latest round of "confidence building" negotiations which opened in Stockholm last week.

It should be remembered, however, that in 1982, a Moscow radio commentator hinted that the United Kingdom could qualify, like France and India, for participation in the manned "Interkosmos" programme — provided it first rejected the siting of US missiles on its soil.

If the possibility of a joint United States-Soviet major space effort goes further than the recent preliminary kite-flying, it seems highly likely that some concession on arms limitation will be demanded by the Soviet side as a prerequisite to participation.

Vera Rich

UK observatories reprieved

GROUND-based astronomers in Britain and particularly those at the Royal Greenwich Observatory (RGO) should be breathing a sigh of relief this week. The Willmore panel set up to advise the Science and Engineering Research Council (SERC) on the future of the British observatories has recommended that RGO, the Royal Observatory Edinburgh (ROE) and Rutherford Appleton Laboratory (RAL) should all continue to be supported by the SERC. This recommendation has been accepted by the body directly responsible for UK astronomy, SERC's Astronomy Space and Radio (ASR) Board, thus stilling fears that RGO might be abolished in the near future (see *Nature* 301, 102; 1983).

The final decision, moreover, rests with the council, due to meet in mid-February, although there is no reason to believe that it will not follow the recommendation. But the observatories will probably not escape totally unscathed, for the plans now laid for instrumentation of the new telescopes at La Palma and Hawaii will probably be

cut back, or be stretched out. Those working at RGO, who have been expecting to co-ordinate the remote operation by satellite of the La Palma telescopes, will for example be displeased by the news that the initial demonstration of this technique, both for La Palma and for the millimetre-wave telescope being constructed in Hawaii, will be carried out at the Edinburgh Observatory.

While these decisions relate to the long-term future of the ASR Board's activities, immediate problems remain. Deficits arising from the effects of exchange rate fluctuations on international subscriptions have been partly compensated for by the Treasury, to the tune of £7 million. But for the financial year 1984-85, the board still has to cut about £2 million from its original estimate. To achieve this, the board intends to cut funds from research grants by 5 per cent, RGO and RAL manpower by about 10 per cent and 15 per cent respectively and other institutional costs by about 10 per cent.

Philip Campbell

India's science defended

SIR — Dr Malviya (*Nature* 306, 10; 1983) says that scientists in India are "not terribly science-minded" and that there is no competition among them. I imagine that, science being universal, "competition" ought to be among the scientists in the world rather than among those of a given country. And if one wishes to compete for recognition in science, one obviously ought to be science-minded.

I believe that Indian scientists working in India are not all that bad. During the past decade at least seven of them have been elected to the Royal Society of London, others as fellows of academies in the United States, the Soviet Union and in European countries while some hold high offices in international scientific unions and the like.

India is a vast heterogeneous nation with pockets of brilliance where the scientists are very good, and also many places of mediocrity, not strikingly different from the spectrum of the quality of Indian expatriates abroad: some very good and some mediocre.

On the hostility of Indian scientists towards their countrymen abroad, the Indian National Science Academy has about 30 of them as fellows in a total membership of some 500, while the Indian Academy of Sciences, in its total fellowship of about 490, has more than 30 Indians settled abroad. And we have not heard of any such hostility from the more than 100 expatriate Indian scientists (Dr Malviya included) who have visited our laboratory at our invitation in the past 2 years.

The question of improving the quality of Indian research necessarily involves the creation and generous support of centres of excellence and this infusion of new blood in its universities and research centres by the creation of additional research chairs with good salaries and facilities. The last idea has already been taken up in the form of a cadre of "research scientist" positions at existing universities. Such positions are open to both residents and expatriates, as they should be. No special consideration for expatriates seems to be necessary.

In considering how to improve the quality of scientific research in India, an appreciation of the existing efforts and problems is essential. Simplistic solutions or blanket condemnations are of no value. I am thus particularly dismayed at Dr Malviya's claim that the people who control science in India are scientifically most incompetent. Those concerned, many of them FRSSs, include those who brought in the green (and now the white) revolution, who have put up Indian satellites and sent Indian research vessels to the Antarctic, set up nuclear plants for power production, trebled the amount of irrigation water in 30 years and controlled communicable diseases.

These scientists advise the central cabinet of ministers on scientific matters.

And it is this scientific competence, utilizing the science and technology base within, that has made India self-sufficient and not a market-place for giant multinational manufacturers.

Let us therefore not tie ourselves into knots of self-flagellation and also keep our temper — the scientific kind — so that we can attempt to solve our problems. (Incidentally, the issue of *Nature* that carries Dr Malviya's letter also carries an article on the cloning of rice embryo histone genes by Indian scientists working in India.)

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Who's playing God?

SIR — In reviewing Paul Davies's *God and the New Physics* (*Nature* 305, 833; 1983) Professor McCrea eschews "any close critical assessment of the work". This approach is akin to that followed by most theologians and practising religionists; to construct elaborate hypotheses based on conjecture and wishful thinking and not squarely to examine the evidence.

While not discounting Dr Davies's scholasticism, it seems a bit over-stated for McCrea to experience "humility" in the light of Davies's "courageous thinking". How courageous must one be, in these times, to espouse ideas dating back to Epicurus? Davies, like many who have preceded him, is merely attempting to intellectualize and dilute the god-concept in order to make it more palatable to those emotionally-weak individuals whose educations would normally preclude such irrational ideas, but who are nonetheless attracted to Universe-schemes which give them a sense of value and purpose other than that which they are able to create for themselves.

This type of accommodation of science by religion has been repeatedly used by the Church throughout the ages in order for it to remain afloat in a sea of scepticism. Any definition of "God" which is interchangeable with "Universe" (another vague term) or "all in all" is completely useless both as a separate concept and as a source of meaning in human existence. Is it not better respectfully to retire such antiquated ideas?

In a decade when science and education are continually being undermined and assaulted by creationists and other pseudo-scientists, it is high time that the scientific community should cease patronizing religion, which we have seen can be quite a formidable opponent to progress and the betterment of the human condition.

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Inviolate usage

SIR — I share the concern indicated by Vimala Sarma, regarding the obfuscation in the recent report of the NH & MRC of Australia, which has adopted a recommendation on rules for fetal research (*Nature* 306, 308; 1983).

Viable, which means capable of maintaining life, is an adjective which may be applied to the embryo, fetus or neonate; for, in the appropriate environment, each is indeed capable of maintaining its life, given adequate oxygen and nutrition, at least. "Previsible" is a term which has no scientific basis, either in embryology or neonatology, to explain a given status of an embryo, fetus or neonate. The report itself indicates as much when it states — "dissection of the fetus should not be carried out while a heartbeat is still apparent or there are other obvious signs of life".

This seems a clear case of scientific jargon obscuring meaning.

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New words for old

SIR — Your offer (*Nature* 306, 134; 1983) to make space available for comment on the abuse of language is welcome. I submit a plea that when new words are coined, they should be unambiguous. It is a pity that the pedantic rule against mixing languages is so often violated. *Prebiotic* may be cited as an example: *probiotic* or *prevital* are preferable.

Some words, for example, *dyke* and *watershed*, have antithetical meanings. In Britain, *watershed* means the line separating catchment areas, and that is the metaphorical meaning in the United States. When used non-metaphorically in the United States, it means a catchment area. So *watershed* is a meaningless word in an international journal. Chemical logic has introduced a few possibilities of ambiguity in written English, although context usually distinguishes the two meanings of such words as *periodic* and *retinal*.

Ambiguity could be avoided if editors took more care to correct the carelessness or ignorance of authors. As T.S. Eliot remarked: "... (words) slip, slide, perish, decay with imprecision ...". We are in danger of losing the distinction between *enormity* and *enormousness*, between *militate* and *mitigate*, and between *suck* and *suckle* (the baby sucks, the mother suckles). The original clearly defined meaning of *visualize* will be lost if the word is stretched to mean to stain or otherwise make visible. Perhaps another word is needed to carry that meaning — I suggest *iconize* or *phanerize*.

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Dispute over scale of Universe

Controversy continues about the scale of the expanding Universe, with the two camps separated by a factor of two. Resolution of the conflict is more urgent than the disputants acknowledge.

Of all the natural constants, Hubble's constant must be the least deserving of the name. Those not directly concerned with the measurement of the distance-scale of the Universe may be forgiven if they harbour the impression that, for the past quarter of a century, the extant value of H_0 has stubbornly refused to settle down within some bounded range of all possible values, eventually to approximate ever more closely to what might be called a true value. Instead, successive estimates of H_0 seem to have fluctuated between two extremes differing numerically by a factor of nearly two.

Behind each value, there is a different school of opinion. Sandage and Tammann, whose latest estimate of Hubble's constant appears in this issue on page 326, are among those who hold that H_0 , the measure of the rate at which the expansion velocity of the Universe increases with distance, is unlikely to be very different from 50 km s⁻¹ per megaparsec (abbreviated to Mpc). The opposing school, represented for example by Dr G. de Vaucouleurs of the University of Texas at Austin, considers that the value of H_0 is more likely to be twice as large, approximately 100 km s⁻¹ Mpc⁻¹. In some ways, the latest contribution from Sandage and Tammann appears to be a direct response to a challenge issued by de Vaucouleurs two years ago (*Nature* 299, 303; 1982), when he argued that a careful examination of the hydrogen-line radio-emission from receding galaxies should provide one of five crucial tests of the conflict between the two distance scales.

This long-standing issue is unlikely, however, to be settled by the appearance of just one more contribution, and neither Sandage and Tammann nor de Vaucouleurs should be blamed for that. For one thing, the problem is uncommonly difficult, as Hubble himself recognized more than half a century ago. Essentially it is that of attempting to construct a distance scale applicable to objects in the Universe so distant that yardsticks usable on smaller scales (for example within our galaxy) are not directly applicable. Instead, they must be translated into secondary yardsticks by processes never free from assumptions about the physical nature of extragalactic objects (and even the space between them).

Then, the problem seems always to become more complicated. While the uniform (in space) and constant (in time) expansion implied by the definition of the

Hubble constant may be a good first approximation to the Universe in the large, only in the past fifteen years has it become plain that we perforce look out at the Universe from an exceptional place, one near enough to the Virgo cluster for the motions of most nearby galaxies (on which previous estimates of distance have largely been based) to be streaming towards its centre of gravity, and even for the motion of our own galaxy (as measured by the anisotropy of the microwave background radiation) to be influenced as well.

Inevitably, these tenuous chains of inference run into serious trouble in the most distant reaches of the Universe, where the galaxies may be physically different (witness the quasars) from those in our immediate neighbourhood. One practical consequence is that people seem for the time being to have abandoned hope (still strong less than a decade ago) of being able to determine accurately from direct observation any but the first linear approximation to the expansion of the Universe. The Hubble constant may be uncertain, but the range of values admissible for the deceleration of the expansion — the next approximation — is so great that the argument whether the Universe is open or closed has reverted entirely to the theorists.

The article by Sandage and Tammann will find a wide hearing simply because it offers a novel test of a sensible prediction of the relationship that should exist between the intrinsic luminosity (or mass) of a galaxy and the maximum velocity of its interstellar gas, predominantly hydrogen. Crudely, the greater the mass within some distance of the centre of a galaxy, the greater will be the gravitational potential at that distance so that, other things being equal, the greater will be the velocity of freely moving material at that point.

So, according to what is known as the *Tully-Fisher* relationship, it should be possible to infer, from the broadening of say the 21-centimetre hydrogen line, the spread of velocities of hydrogen clouds within the galaxy and that, by comparison with other galaxies of the same type, should yield an estimate of the intrinsic magnitude of the distant galaxy. The use of the *Tully-Fisher* relationship is not of course new; two years ago, de Vaucouleurs used exactly such an argument to support his view that the Hubble constant is roughly twice that now quoted by Sandage and Tammann.

How can such discrepancies arise in the analysis of the same data? And how will

they eventually be resolved? The obvious difficulty is that the process of extending the distance scale beyond nearby galaxies is far from being objective. Whatever physical principle is used to help determine the intrinsic brightness of a distant galaxy, people will eventually be driven to select, from among all galaxies for which measurements are available, a sample which can be held to be comparable with each other.

The snag, as the experience of past decades has shown, is that this process of selection can also be a source of bias, involving as it does explicit or even hidden assumptions about the physical reasons why one type of galaxy differs from another. Indeed, these chains of inference are so complicated that many of the minor controversies that arise within the ambit of the larger controversy are discovered, in the end, to have arisen because some set of apparently independent measurements is found to hang on a few or even a single one of them.

The only remedy is the old remedy — more data. In the long run, the construction of a reliable distance scale will require such a detailed study of the properties of the galaxies involved that the propriety of the comparisons made can be verified directly. The search for novel ways of comparing the intrinsic brightness of near and far galaxies should of course continue (as it will), but there should by now be enough experience, most of it disappointing, for the practitioners to know that there is no magic solution on the cards.

What, in the meantime, is to happen to the controversy between the opposing camps, each of which is convinced of the correctness of its own estimates of the distance scale? Sandage and Tammann point out that their own estimate of Hubble's constant has the virtue of suggesting an age for the Universe as a whole which is more nearly in agreement with Astrophysical estimates of the age of the objects which it contains than the shorter time-scale implied by the longer distance scale of de Vaucouleurs and others (which does not, of course, prove that it is correct). This circumstance seems to infuriate the other camp, with the consequence that the controversy is no longer seemly. The ideal would be that those concerned should acknowledge that the crying need is for a reliable distance-scale, not for a proof that one or other of the two candidates now in the field is the better.

John Maddox

Archaeology

Clues to recent human evolution from specialized technologies?

from Marek Zvebil

A WEALTH of new studies of the technology used by hunters and gatherers to procure their food are making it possible to guess what part advances in technology played in human evolution. Particularly interesting are two recent analyses by R. Torrence¹ and R. Dennell², building on earlier work by Binford³⁻⁶, of the way in which the development of specialized stone tool technologies allowed foraging societies dependent on a narrow range of resources to live successfully in cold northern climates. The analyses show that devoting time to the production of special tools may have made possible the replacement of the neanderthal by modern man during the last glaciation, and the colonization of sub-Arctic areas some 30,000 years ago.

In their efforts to survive, prehistoric societies employed various technologies. The most ancient of them — that of stone — has been in use for more than two million years. But the making of tools takes time — time which could be spent on other activities, including the search for food. Benefits derived from the use of any range of tools must be carefully weighed against the time and effort needed to make them, and their manufacturer and the search for raw materials must be organized so that they complement other activities required for subsistence. In favourable environments, where resources are evenly distributed in space and over seasons, people

can usually obtain their food with the aid of only relatively simple technology. Using data collected by Oswalt⁷, Torrence¹ has shown that the use of simple general-purpose instruments and weapons is associated with hunter-gatherer societies that live in tropical and sub-tropical climates, where most of the food comes from stationary reliable plant resources. In contrast, where resources are seasonal and mobile, as in the Arctic and sub-Arctic, highly specialized complex tools are found that require considerable skill and planning to produce.

To exploit seasonal and mobile resources (the characteristics often occur together, as in migratory herbivores, water-fowl, seals or anadromous fish) requires a long time: both to locate prey (search time) and to capture it (pursuit time); but the time available is limited. Foragers can increase the reliability and productivity of their subsistence strategy by using time-saving devices: by budgeting their time and by preparing in advance more specialized but also more complicated tools, designed for each of the number of tasks involved (Fig. 1). In societies dependent on stationary ubiquitous resources, time is not so important and the use of a complex and specialized technology would not bring benefits that would justify the time and effort spent in its development.

In a recent publication, Dennell² linked

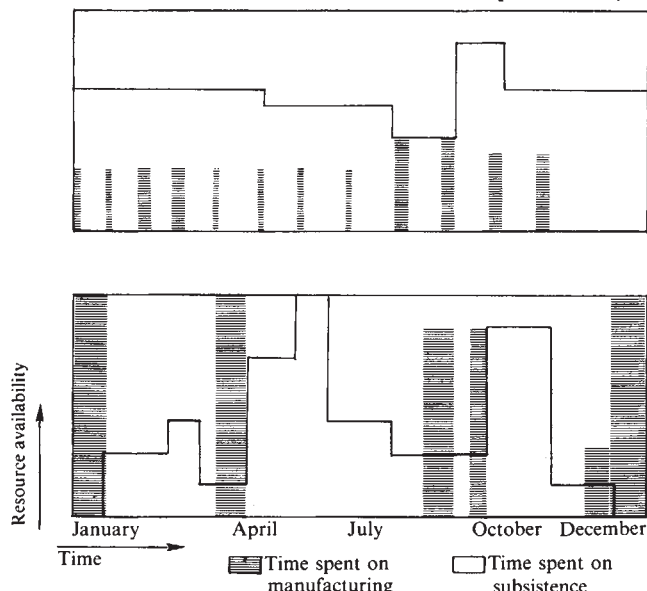


Fig. 1 Tool manufacture and seasonal variation in the level of resources procured. The upper diagram describes a system where permanent stationary resources are exploited using generalized technology (as, for example, in tropical gatherer communities). Little time is spent on manufacturing, which is evenly spread throughout the subsistence activities. The lower diagram shows how seasonal and migratory resources are exploited using specialized technology (for example by boreal hunter-fishers). Here, food procurement time is at a premium. Manufacturing time is greater and is located in periods of minimum subsistence activity.

the technological developments of the Upper Palaeolithic with the conceptual advances which marked the difference between modern man and neanderthals. Compared with the Mousterian — the technology which spanned the middle Palaeolithic — upper Palaeolithic industries were more complex: the number of techniques used for working raw materials increased, as did the range of raw materials; and the number of components making up an artefact increased, as did the diversity of tool types. The number of stages involved in making artefacts increased significantly; according to Dennell² it is this feature that marks the crucial difference between middle and Upper Palaeolithic technologies. The middle Palaeolithic Mousterian was essentially an expedient technology: tools were made for immediate needs in a short sequence of single-stage operations. It is perhaps for this reason that raw materials which required a large amount of time and labour to make into artefacts, such as bone, ivory or antler, were not used extensively in tool making until after 30,000 BP. In contrast, upper Palaeolithic artefacts were made in anticipation of future needs in a series of temporally and conceptually separate stages, employing a variety of different techniques and separate manufacturing processes. This required advances in planning and, possibly, advances in manual dexterity that might have been beyond neanderthals' mental and physical capabilities.

The similarity between the new upper Palaeolithic technology and the technology used by modern foragers in cold environments seems remarkable until we realize that both populations were trying to cope with the same type of time-stressed seasonal environments: the cold temperate and Arctic habitats, where resources tend to be not only seasonal and migratory but also to contain fewer species. These characteristics mutually reinforce each other in encouraging the development of specialized technology: seasonality and

1. Torrence, R. in *Hunter-Gatherer Economy in Prehistory* (ed. Bailey, G.) 11 (Cambridge University Press, 1983).
2. Dennell, R. *European Economic Prehistory* (Academic, London, 1983).
3. Binford, L. in *Stone Tools as Cultural Markers* (ed. Wright, V.S.) 24 (Australian Institute for Aboriginal Studies, Canberra, 1977).
4. Binford, L. *J. anthrop. Res.* 35, 255 (1979).
5. Binford, L. *Am. Antiq.* 45, 4 (1980).
6. Binford, L. *Nunamiut Ethnoarchaeology* (Academic, London, 1978).
7. Oswalt, W.H. *An Anthropological Analysis of Food Getting Technology* (Wiley, New York, 1976).
8. Dennell, R. *Nature, News & Views* 301, 199 (1983).
9. Trinkaus, E. in *Mousterian Legacy* (ed. Trinkaus, E.) (British Archaeological Reports, Int. Ser. 164, Oxford, 1983).
10. Trinkaus, E. & Howells, W.W. *Scient. Am.* 241, no.1, 94 (1979).
11. Brose, D.S. & Wolpoff, M.H. *Am. Anthrop.* 73, 1156 (1971).
12. Klein, R.G. *Quat. Res.* 1 (1971).
13. Mochanov, Yu. A. in *Early Man in America from a Circum-Pacific Perspective* (ed. Bryan, A.L.) (Edmonton, 1978).
14. Morlan, R.G. in *Early Man in America from a Circum-Pacific Perspective* (ed. Bryan, A.L.) (Edmonton, 1978).

mobility because specialized technology saves search and pursuit time; low diversity because specialized technology acts as an insurance against failure.

Organizational changes brought about by the use of specialized technology^{1,5} must have aided upper palaeolithic man considerably to cope with the cold and seasonal environments of the temperate zone during the later part of the last glaciation⁸. In competition with these more flexible cultural adaptations, the physiological adaptations of neanderthal man to cold climate^{9,10} proved inadequate. Assuming that in western Europe we can equate neanderthal man with the old Mousterian technology and modern man with the new — an assumption not universally accepted¹¹ — then the disappearance of neanderthals around 35,000–30,000 BP can probably be attributed to their failure to compete successfully with the better equipped *Homo*

sapiens sapiens for the mobile and seasonal resources of the last ice age.

The observations made by Dennell and Torrence have some further implications. The failure of the neanderthals may illustrate the inability of human populations to survive and prosper in cold and seasonal climates without the aid of complex and specialized technology. There is no evidence of human occupation of Siberia before 35,000 BP, while the earliest reliable evidence for the presence of humans in North America dates to 27,000 BP (refs 12–14). It may be that successful colonization of these sub-Arctic regions (as they then were) could not have been accomplished without the technological and organizational advances attained by modern man. □

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Optical bistability

Towards the optical computer

from S. D. Smith

NEXT month (21 and 22 March) the Royal Society will hold the first European conference devoted exclusively to optical bistability and photonic logic. This is a mark of the growing appreciation of the potential of these devices in the design of new kinds of computers that will not merely be faster than, but different from, the machines now being used.

Sequential binary logic has had success enough in the fields of signal processing, transmission and computing using one-dimensional electronic circuits as to be now accepted as one of the vital elements of our new industrial economies. But this familiar method, because it is sequential and because the signals are confined to a conducting wire, is clumsy and primitive. The disadvantages are overcome, say for a computer calculation or in building up a television picture, only by means of a large number of high-speed switching operations.

The possibility that optical devices might be superior goes back to 1969, when Abraham Szöke and colleagues at MIT put forward a first statement of a principle of optical bistability¹ based on the combination of nonlinearity, represented by saturable absorption, with positive feedback provided by an optical resonator. This led to the simple idea of an optical device consisting of a Fabry-Perot interferometer with an absorbing spacer layer illuminated by a laser and having two stable states — one at low power in which there is no interference and low transmission and one in which, at high power, the spacer absorption is saturated, constructive interference can take place and both transmission and internal intensities in the resonator are large. Once reached, the se-

cond state could in principle be maintained with a lower power than required to achieve it. The output and input of the device are thus related by a bistable loop (Fig. 1).

In reality, the effect was not observed until 1976, when a bistable optical effect was found in sodium vapour at the Bell Laboratories². But analysis showed that the effect was due to refraction, not absorption, almost coincidentally with the publication of a theory of refractive optical bistability³ based on the idea that refractive index can vary with light intensity. In retrospect, it turns out that absorptive bistability is much less easily observed than refractive bistability, basically because of the greater sensitivity of the interferometric effects when a resonator length is tunable through the intensity dependence of refractive index.

The device operates as follows (see Fig. 2). An interferometer is illuminated by a laser at a wavelength slightly detuned from resonance at some intensity (called the 'holding power'). Increase of intensity changes the optical thickness, moving the device towards the resonance. But, as resonance is approached, the ratio of the internal field to the incident field increases as interference becomes more constructive. Because it is the internal intensity that causes the nonlinearity and hence the optical thickness change and the rate of tuning, positive feedback is provided. The rate of tuning increases dramatically and the resonator can switch in a bistable manner.

The intrinsic interest of optically bistable effects caused an outburst of theoretical work, mainly concerned with two-level atomic systems, but the most significant steps towards a potential new technology were perhaps our discovery in 1976 at the

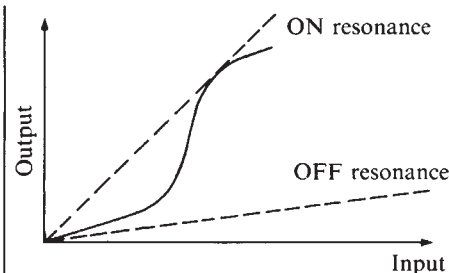


Fig. 1 Schematic relationship between input and output light intensities for a bistable device (full line). The sharp increase of output with input in the middle of the diagram explains how such a device may be used as a fast switching device.

Heriot-Watt University in Edinburgh of giant band-gap resonant nonlinear refraction in a narrow-gap semiconductor⁴ and of similar effects, albeit a thousand times smaller, near the exciton frequency in GaAs by a Bell Laboratories group⁶ in 1979.

The first discovery, giving a nonlinear refractive index n defined by $n = n_0 + n_2 I$ of the order $n_2 = 1 \text{ cm}^2 \text{ kW}^{-1}$, meant that nonlinear optics was transformed from megawatt to milliwatt powers. The origin of this previously unnoticed effect lies in the 'blocking' or saturation effect of promoting electrons into states near the band-gap, with consequent large changes in the real part of the dielectric constant. The development also meant that devices a few micrometres in dimensions could be constructed. Thus in 1979 we⁵ and the group at the Bell Laboratories⁶ simultaneously reported the observation of optical bistability in small semiconductor resonators. Our group at the same time described 'transphaser' (that is, optical transistor) action with a two-beam signal gain of about 10 (see text below and Fig. 6).

These experiments have now set the scene for a complete family of optical circuit elements⁷ (see Figs 3–5). Simply by adjusting the initial detuning, either single-valued characteristics with steps of slope greater than 1 (gain) or bistable loops of variable width can be obtained. Moreover, a system such as InSb at 77K can be held by

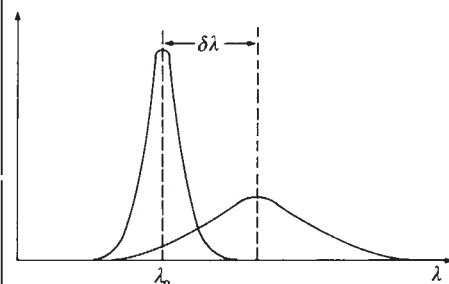


Fig. 2 Change of state of nonlinear refractive optical resonator with increasing light intensity. The refractive index n is $n_0 + n_2 I$, where n_0 and n_2 are constants, and I is light intensity. Increasing intensity increases n and thus reduces the wavelength λ to coincide with a value λ_0 , an integral sub-multiple of the length of the resonator.

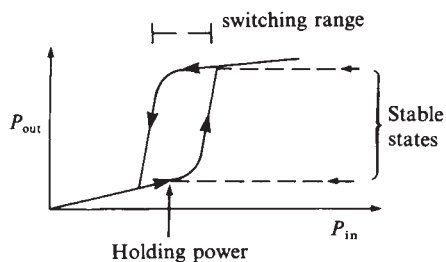


Fig. 3 Bistable optical element as a binary memory device.

a continuous bias beam at a given point on the characteristic and then addressed by a light pulse, and such a system was used in 1982 to demonstrate optical AND/OR and NAND/NOR logic gates using both reflection and transmission⁸.

In the past year, devices that function at room temperature have been made from a variety of systems such as GaAlAs multiple-quantum-well systems (Bell and Arizona, at 0.8 μm), Te (Jena, at 10.6 μm), InSb (Heriot-Watt, at 10.6 μm) and liquid crystals (Berkeley, visible). At the same time, other narrow-gap semiconducting materials have been introduced such as CuCl (Strasbourg, visible), CdS (GTE and Frankfurt), CdHgTe (RSRE) and InAs (University of Southern California) at cryogenic temperatures. The speeds of these devices and the light intensity required vary considerably, and with the present rapid progress, it is too soon to know which materials will prove most suitable for which purpose. But many systems suggest switch-up times of the order of picoseconds. Fast switch-down has yet to be confirmed, but seems possible.

Novel approaches to either the geometry of the feedback process (such as focusing) or the nonlinearity have also recently been pursued. One intriguing development has been the use of radiation pressure⁹ on a freely mounted mirror forming part of a Fabry-Perot device (Max Planck Institute for Quantum Optics, and the University of Munich). In this experiment, the length of the resonating cavity is changed simply by using the radiation pressure impinging on the mirror *in vacuo*. The system is clearly sensitive to vibration and, indeed, has already been recognized as constituting a sensitive seismometer. Direct conversion of acoustic waves to optical modulation (a microphone for optical fibres?) is another possibility.

With the development of this growing family of optical devices based on optical bistability, all the elements required to make an optical computer have now been demonstrated. Where will this lead? If devices based on optical bistability are to compete with those of other switching technologies solely in terms of speed and power, their application would be limited by the continuing advance of silicon technology. But optical devices have qualitative advantages. A lens or mirror can easily communicate thousands of channels of information in parallel, while one op-

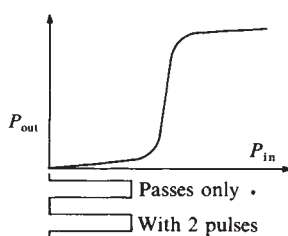


Fig. 4 Optical logic element as an AND gate, giving significant output only when two pulses separately not intense enough to switch states coincide in time and exceed threshold.

tical beam can be passed through another without interference.

By contrast, electronic signals must be individually guided and protected from interacting, and in the design of future electronic computers, switching speeds will certainly increase as very large-scale integration (VLSI) proceeds, but so will the band-width needed for the interaction of components. But communication usually entails some circuit capacitance, and as sizes are reduced, time-constants increase.

In designing a computer architecture to take advantage of the new optical circuit elements, it may well be that interconnection time and the use of parallel channels will be the key points. The 'van Neuman bottle-neck', perhaps the fundamental limitation of digital computers, is the trade-off time for interconnections needed to make up for the inability of electronics to support multiple communications channels in parallel. These are the processes that limit the time taken for a computer to perform a given task.

At this stage, only general statements can be made about the form which an optical computer may take. One of the concepts being investigated, for example, is a two-dimensional array of optical NOR gates with optics, including prisms and lenses, capable of offsetting an output image of the NOR gate array by one pixel and then feeding the output image into the input. More prosaically, if the performance of optically bistable devices at low power

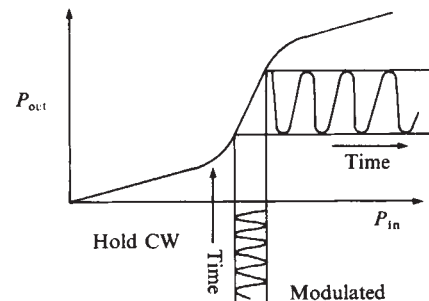


Fig. 5 Transphaser (signal amplifier) in which optically bistable element is held at linear part of input-output relationship. The gain of this device is given by the slope of the curve and may be as great as 10,000 (see text).

levels presently obtained in the infrared can be reproduced with suitable materials in the visible spectrum, technically simple optically-addressed display technology would become available. Power limiters for laser pulses are certainly a practical proposition and, since the most recent work at Edinburgh has demonstrated a transphaser gain of 10,000, all-optical repeaters for fibre communication lines may be possible. Further, incoherent light, which could be in the form of an image, has been shown to cause bistable switching and amplification strongly suggesting that the technique will be useful in image processing. □

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1. Szöke, A., Dancu, V., Goldhar, J., & Kurnit, N.A. *Appl. Phys. Lett.* **15**, 376 (1969).
2. Gibbs, H.M., McCall, S.L. & Venkatesan, T.N.C. *Phys. Rev. Lett.* **36**, 1135 (1976).
3. Felber, F.S. & Marburger, J.H. *Appl. Phys. Lett.* **28**, 731 (1976).
4. Miller, D.A.B., Mozolowski, M.H., Miller, A. & Smith, S.D. *Opt. Commun.* **27**, 133 (1978); for earlier reference to the experimental discovery, see Ironside, C.N. thesis, Heriot-Watt Univ. (1978).
5. Miller, D.A.B., Smith, S.D. & Johnston, A. *Appl. Phys. Lett.* **35**, 658 (1979).
6. Gibbs, H.M. *et al.* *Appl. Phys. Lett.* **35**, 451 (1979).
7. Abraham, E. & Smith, S.D. *Rep. Prog. Phys.* **45**, 815 (1982).
8. Seaton, C.T., Smith S.D., Tooley, F.A.P., Prise, M.E. & Taghizadeh, M.R. *Appl. Phys. Lett.* **42**, 131 (1983).



100 years ago

GEOGRAPHICAL NOTES

THE first report of Prof. Hull, dated from Gaza, January 1, has been received. It is necessarily brief, the details being reserved for the full report to follow, but it announces the success of the expedition so far. The professor has made a complete geological survey of the Wady Arabah and the Dead Sea, with a traverse across Southern Palestine. Capt. Kitchener, R.E., who

accompanied him, has made a trigonometrical survey. Akabah he found to be laid down too far south; the south part of the Dead Sea as shown on the maps, is quite out of its true shape and position, and the Lisan has to be shifted three miles. From Gaza, when the rest of the party were in quarantine, Capt. Kitchener rode back to Egypt, accompanied by four Arabs only. He took a previously unknown route, particulars as to which will follow, and arrived at Ismailia after a ride of 200 miles. He was everywhere well received by the Arabs, who took him for a cousin of Sheikh Abdullah (the late Prof. Palmer), whose memory is still revered among them, and whose murder they still deplore. They are also reported to be deeply impressed with the energy and pertinacity of Sir Charles Warren's pursuit of the murderers.

From *Nature* 29, 318; January 31, 1884.

Immunology

Mechanism of T-cell tolerance

from Roger B. Taylor

In spite of the burgeoning literature on suppressor cells and immunological networks, there has been persistent support for the notion that responsive lymphoid cells may be specifically deleted. This term is now broadly interpreted to mean some internal change occurring in the cell to render it either temporarily or permanently inactive.

We already know that B cells, especially in their immature stages, can enter a condition of lasting unresponsiveness — sometimes accompanied by a defect in the expression of receptors. So far, however, the evidence for deletion of helper T cells has been restricted to experiments *in vivo*, almost entirely in mice, using mammalian serum proteins in high dosage as tolerogens.

To be certain that no suppressor influences are at work one should ideally study tolerance in monotypic cell populations, and preferably clones, *in vitro*. It was therefore a breakthrough, important in more than one sense, when a group working at University College, London, recently achieved the induction of tolerance in human helper T-cell clones *in vitro*¹.

These clones were specific for peptides derived from the influenza haemagglutinin molecule, and were propagated by stimulation with the specific peptide in the presence of unseparated human mononuclear cells which served as antigen-presenting cells. After such stimulation the cells would both exhibit a proliferative response and help B cells to produce antibody. Lamb and his co-workers then tried incubating the clones with high concentrations of the peptides, in the absence of the human mononuclear cells. On subsequently testing the response to the usual low stimulatory concentrations in the presence of the human mononuclear cells they found that the T cells had become specifically unresponsive. The tolerized cells would continue to divide in the presence of interleukin-2 but only for a period of 1–2 weeks, after which their viability declined. A critical finding was that the 'tolerized' cells had lost the surface marker T3 which is thought to be closely associated with the antigen receptor². It confirmed that some demonstrable change had occurred in the cells, and thus argued that the tolerance was not due merely to blocking of the receptors by the high concentration of peptide. A parallel finding was made by Schlossman's group in Boston, who induced a similar state of deletion in human T cells by means not of antigen but of anti-receptor antibodies³.

Against this background, interest now focuses on the mechanism by which such deletion comes about. A pertinent report has recently appeared from Schwartz's

group at the National Institutes of Health, on cloned mouse T cells⁴. These clones, specific for pigeon cytochrome *c*, were propagated by stimulation with this antigen on irradiated mouse spleen cells or a B-lymphoma cell line as antigen-presenting cells. The proliferative response was measured in the presence of varying concentrations of both antigen and the relevant Ia. (The latter was varied by changing the numbers of presenting cells, blocking with anti-Ia antibody, or by the choice of cells homozygous or heterozygous for the relevant Ia.) Bell-shaped curves were obtained, in which the response was inhibited at high concentrations of either antigen or Ia or of both. A clear relationship emerged whereby the response depended on the product of the concentrations of antigen and of Ia. One implication is that a physical product of these two molecules must be the critical driving force for T-cell activation. For the high-dose inhibition to be classed as tolerance, it would need to render the cells refractory to subsequent stimulation with optimal doses. This has not yet been reported. Nevertheless the similarity to the human T-cell work is obvious.

The question now arising is how the T cell is inhibited. Does the occupation of a high proportion of receptors release a negative signal to the cell interior? Or does intense stimulation cause the cell to release critical amounts of an external feedback signal? The authors suggest that immune interferon could serve as such a feedback

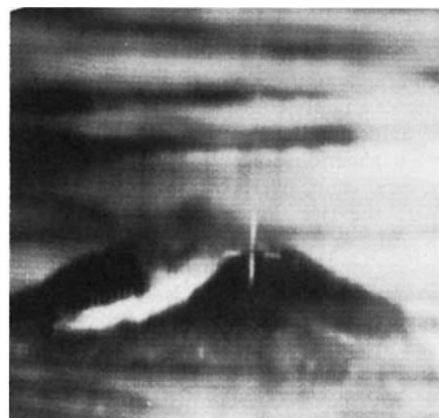
control — particularly as its production is not subject to the same degree of inhibition at high doses of antigen as the proliferative response⁵. There is no evidence so far, however, that the inhibitory effects of interferon on immune responses can persist long enough to be classed as tolerance.

Interferon can also enhance immune responses, as exemplified by a paper in this issue of *Nature*⁶ (see p.381). It is shown that interferon both increases antibody responses *in vivo*, and also increases the expression of Ia on an antigen-presenting macrophage cell line. The authors suggest that the effect of interferon on the antibody response is achieved by virtue of its effect on Ia expression. If this is the case, then the bell-shaped curve derived by Schwartz and colleagues leads us to expect that, at high concentrations of antigen, interferon should aid the induction of tolerance rather than immunity. Its activity might then be seen as to shift the curve to the left, and so influence the quantity of signal delivered via the receptors rather than acting itself as a negative signal. This might also be the role of a variety of other 'adjuvant' influences, many of which can have either positive or negative effects in different circumstances. □

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1. Lamb, J.R., Skidmore, B.J., Chiller, J.M. & Feldmann, M. *J. exp. Med.* **157**, 143 (1983).
2. Lamb, J.R. & Feldmann, M. *Nature* **300**, 456 (1982).
3. Meuer, S.C. *et al. J. exp. Med.* **157**, 705 (1983).
4. Matis, L.A., Glimcher, L.H., Paul, W.E. & Schwartz, R.H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6019 (1983).
5. Hecht, T.T., Longo, D.L. & Matis, L.A. *J. Immun.* (in the press).
6. Nakamura, M., Manser, T., Pearson, G.D.N., Daley, M.J. & Geffer, M.L. *Nature* **306**, 381 (1984).

Latest view of Mount St Helens



MOUNT St Helens photographed during its eruption on 18 May 1980 in normal light (top), and in a recently-released US Navy infrared image. White areas on the infrared image represent hotter areas of the volcano and allow the lava flow near the crater and thermal events not usually seen by the naked eye to be detected. The infrared image was taken from a Hughes A-6E Intruder aircraft using its 'Detecting and Ranging Set', a thermal imaging sensor which, when not photographing volcanoes, helps the Intruder's aircrew see and attack surface targets at night or through smoke and haze. □

Molecular biology

Genetic noise in evolution?

from Darryl Reanney

In 1949 Shannon¹ published an important theorem which states, in essence, that information cannot be transmitted over long periods of time without experiencing some deterioration in quality due to 'noise' in the copier process. Nucleic acids obey this general law as the information they encode is degradable by errors in the copier machinery or by agents such as heat, radiation and mutagenic chemicals².

How important has noise been in evolution? The error frequency in DNA replication in modern eukaryotes is about one incorrect nucleotide incorporated for every 10^9 – 10^{11} bases polymerized during any one round of replication³. But studies on non-enzymatic synthesis in simulated prebiotic environments suggest that the error rate in the 'first' genes was of the order of 10^{-1} – 10^{-2} substitutions per base per generation^{4,5}. Thus as we go back in evolution the amount of noise in the genetic copier mechanism rises by a staggering factor of one hundred million or more.

The amount of information a self-reproducing system can stably maintain is inversely proportional to the error rate of the copier system⁵. Since the accepted mutation rate of enzymatically catalysed polynucleotide synthesis in the absence of repair is about 10^{-3} – 10^{-4} (refs 5,6) one can predict that the maximum amount of information encodable in a single genetic molecule at this stage of evolution was of the order of 10^5 nucleotides⁵ (in point of fact it was probably somewhat less because such a system could not correct errors introduced by external factors such as heat, radiation or pH). In this kind of situation selection would almost certainly favour systems with multiple copies of their genomes since redundancy makes it possible to mask the deleterious effects of harmful mutations by complementation⁷. Thus I believe we can be reasonably confident that early genetic systems were 'polyploid' in the sense that their genetic information was represented several times over. This contrasts sharply with the traditional view that early cells were haploid.

Self-replicating error-prone genomes are today found only in the RNA viruses and of these only the retroviruses possess the genome redundancy postulated to have been a key feature of early genetic systems. Retroviruses are important however because they offer a significant contemporary insight into the influence of noise in evolution. Viruses of this group contain a reverse transcriptase enzyme which makes DNA on a RNA template. Reverse transcription is the only surviving mode of DNA synthesis which is about as error-prone as RNA synthesis⁸. On passing from single-stranded RNA (the free virus) to double-

stranded DNA (the integrated nuclear copy) retrovirus information experiences a millionfold drop in noise levels (see the figure). When this information is transcribed back into RNA the error rate rises by a comparable factor because DNA-dependent RNA synthesis lacks corrective functions. It is difficult to believe that these passages between low- and high-fidelity copier systems have not affected the evolutionary success of retroviruses. When integrated into cellular DNA the viral genes can exploit the repair systems of their host chromosomes and thus maintain strict homogeneity of nucleotide sequence during copying. On re-entering an independent life, the virus can offer a spread of variant sequences (that is survival options) to the test of selection. Retroviruses have the best of both worlds.

The integrated genomes of retroviruses have some tantalizing similarities to certain families of repeated sequences in the mammalian genome such as the *Alu* family⁹ and the genes encoding some small nuclear RNAs^{9,10}. Thus it is not surprising that these families show evidence of having passed through a RNA phase followed by reverse transcription and re-integration into chromosomal DNA^{9,10}. The same seems to be true of 'pseudogenes' — partial duplicates of structural genes, many of which lack introns, have a 3' poly(A) 'tail' and generally resemble mRNAs that have been reverse-transcribed back into DNA. The discovery of pseudogenes for mouse α -globin, human α immunoglobulin, human β -tubulin, human metallothionein and rat α -tubulin⁹ suggests that potentially any sequence in a higher-cell genome can pass through a RNA $\rightarrow$ DNA pathway. Reverse transcription thus closes the flow of information in higher cells into a loop which causes genes to alternate between low- and high-error copying systems.

Could such a cycling of information make a significant contribution to evolution? In assessing this question it is important to recognize the amount of RNA variation that is available. Since transcriptional copying in modern cells retains the high error rate (10^{-3} – 10^{-4}) characteristic of uncorrected nucleic acid synthesis (Fig.1) most transcripts of nuclear genes may contain at least one error. The amount of sequence polymorphism present may be magnified by the ability of the RNA processing mechanism to direct appropriately miscopied RNAs into a variety of alternative splicing pathways¹¹. Given that a single gene in a differentiated cell may generate 50,000 copies of its cognate mRNA¹² it follows that the population of RNA molecules in a clone of cells can experience in a matter of months a degree of

sequence variation which their DNA genome in the absence of selection might take hundreds of thousands of years to achieve. The key question is whether such variations can find their way back into the genome?

Two known mechanisms are available. A processed gene may 'donate' its RNA-based mutations to its parent gene by gene conversion, a process which seems to take place rather frequently in higher cell genomes¹³. Indeed, if a cDNA reverse-transcribed from mRNA were matched against its original gene, then conversion in one direction could imprint the restructured intron-deficient or intron-lacking sequence into its genomic homologue while leaving the promotor signals intact. This would allow processed information to feed back into the genome in functional form. It is also possible that RNA molecules transcribed from transposon-like elements may be reverse-transcribed into a heterogeneous pool of 'nomadic' plasmid-like DNA rings as has been observed in the case of the *Alu* family of sequences¹⁴. These DNA rings would be potentially genetically transmissible through the germline in a non-mendelian fashion without necessarily integrating into chromosomal DNA. Thus cells clonally derived from a common ancestor may become polymorphic in those molecules which have passed through an RNA phase while retaining rigorous sequence identity in the original DNA genes. In this way new adaptive possibilities could be canvassed without erasing the existing phenotype.

Given that effective mechanisms for promoting the RNA-to-DNA pathway exist it is more difficult to assess how often this route of information transfer has been used in evolution. It has been suggested that perhaps 20 per cent of the mammalian genome originated as RNA that was written back into DNA¹⁰. In many systems the figure may be higher. If one assumes that pseudogenes with 15–30 per cent divergence have been seen or inferred for 10 per cent of genes one can say there has been a reintegration event in the germline for about 10 per cent of genes within the last 10–20 million years. This corresponds to about 0.5 per cent to 1 per cent of genes per million years or once for each gene every 100–200 million years. On the other hand it must be admitted that there is no hard evidence that any functional eukaryotic genes are derived from reintegrated mRNAs since known higher-cell genes (with the exception of the α and β interferons and the histones) contain introns. To date the most likely candidate for a one-way gene conversion event is the rat insulin gene which has two alleles, one of which contains two introns while the other contains only one¹⁰.

While there is no evidence for active 're-integrated' genes in eukaryotes it seems likely that the passage of information through the DNA $\rightarrow$ RNA $\rightarrow$ DNA loop has been a factor in the modular evolution

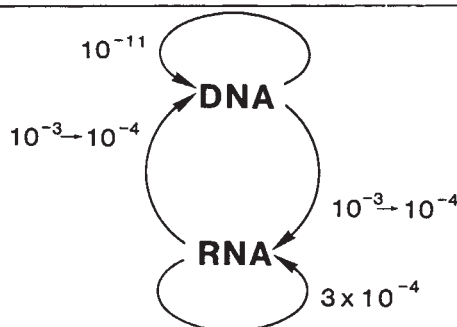


Fig. 1 Information cycles in genetic systems. The error-prone DNA $\rightarrow$ RNA step occurs at high levels in active cells during the normal course of protein synthesis. The error-prone RNA $\rightarrow$ DNA step occurs as an essential part of the life cycle of retroviruses. There is now good evidence that cellular sequences may also follow this pathway on occasions. If one assumes that pseudogenes with 15–30 per cent divergence exist for about 10 per cent of genes one can estimate that reverse transcription of a cellular sequence followed by re-integration occurs in the germ line once for each gene every 100–200 million years. This is slow but significant on the evolutionary time scale.

The actual rate may of course be much higher.

of split genes. Blake and others have suggested that exons correspond to discrete peptide substructures which often have specific functions^{15,16}. Each time a mosaic gene (in whole or part) cycles through an RNA intermediate back into the genome, intron removal knits the exons together into more complicated structures. Such multi-exon aggregates would normally be inactive but they could regain activity by conversion, or by incorporation into other transcriptional units followed by mutation. Such processes would, over evolutionary time, increase the complexity of protein structure by building more and more functional capacities into the one polypeptide. On this basis older genes would consist of very short exons embedded in an excess of 'junk' while modern genes would contain fewer introns and more complex exons.

Many contemporary genes bear the stamp of this kind of process. According to Go, the globin gene, which has two introns, is made up of four peptide modules, not three (see ref. 17). Gō believes the now contiguous central coding region of the gene was once split by an intron¹⁷ which has since been precisely removed, presumably by passing through an RNA processing stage. Similar processes seem to have occurred in the genes for egg white lysozyme

and cytochrome *c*¹⁷. The end products of this bit-by-bit process of protein evolution are the uninterrupted genes of prokaryotes, where, according to Doolittle¹⁸ and others, all introns have been removed in the interests of efficiency and economy.

Apart from its possible relevance to protein evolution the ability of nuclear infor-

mation to occasionally pass through a noisy RNA copier has implications for students of evolution who seek to estimate times of (recent) evolutionary divergence by comparing the base sequence of an intron-lacking pseudogene with that of its intron-plus parent (see for example ref. 19). Such calculations should be tempered by the knowledge that a molecular clock based on RNA runs at a much faster rate than one based on DNA.

While DNA is a high-fidelity copier system this very stability may deny it a preponderant role in short-term adaptation. By contrast the noisiness which makes RNA a genetic liability (where 'large' genomes are concerned) also makes it an ideal channel for the controlled introduction of novelty. In a limited but important sense then DNA may be regarded as the guardian and RNA the modifier of genetic identity. By switching back and forth between these extreme levels of noise evolution may have struck an effective balance between conservatism and change. □

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Cell patterning

Plants under strain

from Clive Lloyd

BEING nasty to your houseplant might put it under strain but, for a plant developmental biologist, the word strain takes on a different meaning: it denotes those tensions, propagated across cells and tissues, that have been hypothesized to orientate the insertion of new cross-walls, and so play a part in cell patterning. Scientists have been trying for some time to come to terms with the forces that influence the partitioning of cells in tissues. Observation of groups of cells can persuade that purely physical rules are involved and nineteenth century scientists (and, later D'Arcy Thompson) compared the behaviour of young cross-walls, of which no more than three walls are seen to meet at one point, to liquid films. But bricks, rather than bubbles, provided the analogy for Sinnott and Bloch's pioneering work in the 1940s.

They noted that when a new cross-wall formed in one file of cells, it avoided lining up with a cross-wall in the neighbouring file — much as bricks are staggered between courses in a wall. However, they also recorded that there was no such avoidance in cells induced to divide by wounding; thereby illustrating that previously formulated 'rules' are frequently broken^{1,2} because the quality of the response seems to be affected by local conditions. Other examples of 'rule breaking' appeared. New cell walls may initially be formed parallel to the sides of the mother cell but, upon

further centrifugal development, the growing cell plate can swerve towards one side, producing a smaller wedge-shaped cell — from a purely physical analogy it could be argued that the new cross-wall should be of the minimum surface area required to divide the cell.

The lasting contribution that Sinnott and Bloch³ made to plant histogenesis was to emphasize that in vacuolate cells, a plate of cytoplasmic strands becomes positioned across the vacuole, with the ends of the strand attached to the mother cell walls along the line where the new cell plate will join them, before even mitosis has taken place. This structure, the phragmosome, can lay claim to the title of being the organelle least understood yet most likely to help our understanding of pattern formation — the task now being to find out more about its structure and about the forces that orientate it.

Forces that orientate the new cell plate are the subject of a letter in today's *Nature* by Lintilhac and Vesecky (see p.363). The point they make is that extracellular mechanical cues are sensed by cells in tissues and the new cell plate is orientated so that it is maximally protected from shear. Lintilhac and Vesecky studied tobacco pith, which, when taken into culture, grows into a disorganized callus. They attempted to re-establish ordered patterns of division by compressing callus in a frame, on the grounds that the *in vivo* mechanical

- Shannon, C.E. in *The Mathematical Theory of Communication* (eds Shannon, C.E. & Weaver, W.) (University of Illinois Press, Urbana, 1949).
- Drake, J.W. *Symp. Soc. gen. Microbiol.* **24**, 41 (1974).
- Kornberg, A. *DNA Replication* (Freeman, San Francisco, 1980).
- Inoue, T. & Orgel, L.E. *Science* **219**, 859 (1983).
- Eigen, M. & Schuster, P. *Naturwissenschaften* **64**, 541 (1977).
- Domingo, E., Sabo, D., Taniguchi, T. & Weissman, C. *Cell* **13**, 735 (1978).
- Reaney, D.C., MacPhee, D.G. & Pressing, J. *Aust. J. Biol. Sci.* **36**, 77 (1983).
- Battula, N. & Loeb, L. *J. biol. Chem.* **250**, 4405 (1975).
- Sharp, P.A. *Nature* **301**, 471 (1983).
- Lewin, R. *Science* **219**, 1052 (1983).
- Mount, S. & Steitz, J. *Nature* **303**, 380 (1983).
- Long, E. & Dawid, I. A. *Rev. Biochem.* **49**, 727 (1980).
- Dover, G. *Nature* **299**, 111 (1982).
- Shmookler Reis, R. *et al. Nature* **301**, 394 (1983).
- Blake, C.C.F. *Nature* **277**, 598 (1979).
- Inana, G. *et al. Nature* **302**, 310 (1983).
- Lewin, R. *Science* **217**, 921 (1982).
- Doolittle, W. *Nature* **272**, 581 (1978).
- Icmischka, I. & Sharp, P.A. *Nature* **300**, 330 (1983).

environment for growth has been lost and cannot be restored by hormonal manipulation alone. After five days under compression, co-planar divisions were found in sections of calli. Allowing for the difficulty of obtaining uniform results from such variable material as callus it is intriguing that compression can yield co-planar divisions up to a depth of ten ranks of cells.

The observation will add fuel to an idea expressed at different times in different ways (see, for example, refs 4–7) that the development of plant form involves epigenetic control over the alignment of cross-walls through a programme of division and expansion which imparts each cell with a different positional value that is 'read' in terms of strain alignment of the cell plate.

At the cellular level, however, it is still not understood how the cell plate is aligned. Vacuolate cells, stimulated to divide by wounding the tissue, possess a circumferential band of microtubules⁸ whose orientation forecasts the plane of division in much the same way that the pre-prophase band of microtubules does in meristematic cells (for a review, see ref. 9). However, the question of whether the pre-prophase band is the result or the cause of cytoplasmic polarization remains unresolved, although recently published observations by Galatis *et al.*¹⁰ throw interesting light upon it.

In the stomatal complex of *Triticum* leaves, two pre-prophase bands, rather than one, are sometimes formed simultaneously within a single long cell. Following mitosis, a cell plate is deposited which curves from one of the cortical zones, previously marked by a pre-prophase band, to the other. Again, this behaviour is contrary to the minimal surface area hypothesis for cytokinesis. It is not known whether such curved cell plates are orientated by chemical or mechanical signals, but the observation that pre-prophase and telophase nuclei form acute local extensions (which these investigators liken to the pulling of the nucleus) hints at mechanical involvement. Unfortunately, all we have at the moment is hints; physical connections between nucleus and cortical cytoplasm are not yet resolved in higher plants but, if real, may provide a fuller understanding of how the growth of plant cells is patterned. □

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- Sinnott, E.W. & Bloch, R. *Am. J. Bot.* **28**, 607 (1941).
- Sinnott, E.W. *Torreyia* **43**, 29 (1943).
- Sinnott, E.W. & Bloch, R. *Proc. natn. Acad. Sci. U.S.A.* **26**, 223 (1940).
- Sobota, A.E. & Partanen, C.R. *Can. J. Bot.* **44**, 497 (1966).
- Cooke, T.J. & Paolillo, D.J. *Am. J. Bot.* **67**, 1320 (1980).
- Miller, J.H. *Am. J. Bot.* **67**, 534 (1980).
- Lintilhac, P.M. & Vesecky, T.B. *Am. J. Bot.* **68**, 1222 (1981).
- Venverloo, C.J., Hovenkamp, P.H., Weeda, A.J. & Libbenga, K.R. *Z. Pflanzenphysiol.* **100**, 161 (1980).
- Gunning, B.E.S. in *The Cytoskeleton in Plant Growth and Development*. (ed. Lloyd, C.W.) 228 (Academic, New York, 1983).
- Galatis, B., Apostolamos, P. & Katsaros, C. *Protoplasma* **117**, 24 (1983).

Astronomy

IRAS circular 6

The source name consists of four parts: (1) the letters 'IRAS' to indicate the origin; (2) the right ascension (RA) in hours and minutes, seconds omitted; (3) declination (Dec) in decimal degrees, multiplied by 10 and then truncated (thus +32° 42.3 becomes +327); (4) an appendix starting with 'P' and followed by the number of the circular; this appendix stresses that the data are preliminary. Position is given at Equinox 1950.0. The measurements were made between epochs 1983.1 and 1983.6. Sources labelled PO3 were published in IRAS circular no. 3 and are included for completeness.

Source IRAS	RA (1950) h min s	Dec (1950) deg arc min s	Identification	12 μ m	Flux density (Jy) 25 μ m	60 μ m	100 μ m
0125+848P03	01 25 27.9	+84 45 11	U1039	<0.2	<0.2	0.50	2.3
0353+261P06	03 53 19.8	+26 05 54		<0.4	<0.4	0.52	<1.5
0355+184P06	03 55 19.3	+18 26 32		<0.2	<0.2	0.62	<1.2
0356+202P06	03 56 05.1	+20 11 56		<0.7	<0.3	0.57	<1.7
0356+217P03	03 56 33.0	+21 39 16	ZW487.004	<0.2	<0.2	0.76	1.9
0357+209P06	03 57 46.7	+20 54 40	M+03-11-004	<0.4	<0.3	0.65	<1.2
0359+140P06	03 59 50.6	+14 01 32		<0.4	<0.2	0.71	<1.6
0400+248P06	04 00 53.5	+24 46 35		<0.3	<0.3	0.80	<1.4
0402+212P03	04 02 19.2	+21 14 20		<0.2	<0.2	1.22	2.6
0402+112P06	04 02 52.1	+11 10 03		<0.2	<0.3	0.77	<2.3
0406+085P03	04 06 29.9	+08 31 05	U2970	<0.2	<0.2	0.95	4.8
0409+054P03	04 09 42.2	+05 25 08	U2982	0.55	0.79	9.3	20.8
0412+064P06	04 12 04.3	+06 22 10		<0.3	<0.2	0.70	<1.4
0413+081P03	04 13 24.3	+08 03 29	U2997	<0.2	0.68	5.37	7.0
0413+026P06	04 13 57.3	+02 38 02	U2998	<0.2	<0.2	0.74	3.2
0414+011P03	04 14 07.3	+01 03 35		<0.2	<0.2	0.77	2.3
0414+001P03	04 14 11.0	+00 09 01		<0.2	<0.39	1.99	4.0
0414+103P03	04 14 28.9	+10 20 04		<0.2	<0.3	3.14	8.5
0414+047P06	04 14 36.8	+04 39 38	U3003	<0.2	<0.2	0.65	1.8
0414+023P06	04 14 43.2	+02 18 47	U3004	<0.2	<0.2	1.51	5.1
0415+014P06	04 15 05.3	+01 26 08	2ZW007	<0.2	<0.4	3.05	6.6
0416+031P03	04 16 12.9	+03 06 33		<0.2	<0.2	0.75	2.9
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0417-011P06	04 17 30.4	-01 06 51		<0.2	<0.2	0.82	2.7
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0418+058P06	04 18 48.4	+05 48 32		<0.2	<0.2	0.49	2.1
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0419-009P06	04 19 26.6	-00 55 31	U3022	<0.2	<0.2	0.53	1.9
0420+044P06	04 20 24.2	+04 25 48		<0.2	<0.2	0.51	2.1
0421+040P06	04 21 01.4	+04 01 00		<0.2	0.35	0.68	<1.8
1648-024P06	16 48 47.0	-02 22 15	U10578	<0.2	<0.3	2.67	6.2
1650-022P06	16 50 08.1	-02 10 11		<0.2	<0.2	0.50	<1.5
1653-012P06	16 53 23.7	-01 10 18		<0.2	<0.3	4.08	8.2
1654+030P06	16 54 42.6	+02 57 35		<0.2	<0.2	1.76	2.2
1657+026P06	16 57 15.0	+02 35 02	U10640	<0.2	<0.2	0.58	<1.5
1658-018P06	16 58 22.6	-01 46 29		<0.2	<0.4	0.52	1.6
1658+069P06	16 58 42.7	+06 55 49	ZW053.023	<0.1	<0.3	1.41	1.8
1658+054P06	16 58 54.2	+05 21 19	ZW053.024	<0.2	<0.2	2.22	4.6
1700+770P06	17 00 19.9	+77 02 26		<0.2	<0.2	0.56	1.8
1701+043P06	17 01 15.8	+04 18 53	ZW053.027	<0.2	<0.2	0.55	1.3
1701+030P06	17 01 31.8	+03 00 23		<0.3	<0.2	0.73	<1.2
1702+772P06	17 02 00.5	+77 14 17		<0.2	<0.2	0.61	1.4
1702+100P06	17 02 36.9	+09 59 47	M+02-43-006	<0.2	<0.2	0.57	2.5
1702+081P06	17 02 44.1	+08 03 26		<0.2	0.45	2.48	3.9
1703+104P06	17 03 56.9	+10 26 28	U10699	<0.2	<0.2	2.25	6.0
1704+066P06	17 04 06.5	+06 36 15		<0.2	<0.2	0.50	2.3
1706+041P06	17 06 14.1	+04 06 45	U10718	<0.2	<0.2	0.86	2.6
1709+081P06	17 09 06.7	+08 03 13	U10743	<0.2	<0.2	3.39	4.3
1710+166P06	17 10 10.0	+16 37 15	ZW111.012	<0.2	<0.2	0.48	2.0
1711+129P06	17 11 45.7	+12 53 33		<0.2	<0.2	0.71	4.9
1711+788P06	17 11 56.0	+78 49 56		<0.3	<0.2	0.45	<1.4
1715+197P06	17 15 15.5	+19 40 17		<0.2	0.44	2.33	4.8
1715+117P06	17 15 25.1	+11 41 26		<0.2	<0.2	1.80	3.8
1715+126P06	17 15 37.5	+12 38 12		<0.2	<0.2	0.93	<1.1
1717+167P06	17 17 40.5	+16 42 43	U10807	<0.2	<0.2	0.72	2.9
1721+212P06	17 21 51.6	+21 10 53	ZW140.018	<0.2	<0.3	2.37	5.6
1722+191P06	17 22 22.5	+19 06 43		<0.3	<0.2	0.66	<1.1
1725+211P06	17 25 20.3	+21 08 34		<0.2	<0.2	0.91	2.3
1730+202P06	17 30 00.6	+20 09 39		<0.2	<0.2	0.50	1.7
1733+803P06	17 33 00.9	+80 16 34		<0.2	<0.2	0.77	1.8
1735+263P06	17 35 18.4	+26 16 25	ZW140.055	<0.4	<0.2	0.52	<1.5
1736+250P06	17 36 23.9	+24 58 54	U10926	<0.2	<0.2	1.20	2.5
1737+287P06	17 37 46.6	+28 44 59		<0.2	<0.2	0.62	1.7
1738+291P06	17 38 41.4	+29 08 45		<0.3	<0.2	0.67	<1.0
1740+256P06	17 40 01.3	+25 38 27	M+04-42-002	<0.2	<0.2	1.16	2.8
1744+307P06	17 44 33.9	+30 43 17	U10976/7	<0.2	<0.2	1.92	6.1
1751+319P06	17 51 21.1	+31 53 00		<0.4	<0.2	0.61	<1.4
1751+339P06	17 51 55.8	+33 51 20		<0.2	0.39	1.40	2.6
1752+329P06	17 52 39.2	+32 53 34	U11035	<0.3	<0.42	3.59	9.2
1753+348P06	17 53 04.3	+34 47 02	U11041	0.34	0.74	6.38	16.8
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1803+338P06	18 03 55.8	+33 49 28		<0.2	<0.2	0.67	1.9
1803+347P06	18 03 57.5	+34 44 48	M+06-40-003	<0.2	<0.2	0.65	2.1
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1805+356P06	18 05 40.9	+35 33 27	U11124	<0.2	<0.2	0.60	1.9
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1813+373P06	18 13 01.5	+37 20 44		<0.2	<0.2	0.83	1.9
1816+398P06	18 16 28.3	+39 48 00	M+07-37-036	<0.2	<0.2	0.89	3.1
1820+416P06	18 20 17.1	+41 33 14		<0.2	<0.2	0.49	6.1
2126+871P06	21 26 16.8	+87 05 13		<0.2	<0.2	0.63	<1.7
2327+853P06	23 27 02.0	+85 18 34	7ZW941	<0.2	0.2	1.81	6.1

The complexity and stability of ecosystems

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Early studies suggested that simple ecosystems were less stable than complex ones, but later studies came to the opposite conclusion. Confusion arose because of the many different meanings of 'complexity' and 'stability'. Most of the possible questions about the relationship between stability-complexity have not been asked. Those that have yield a variety of answers.

ELTON¹ noted the dangers of human simplification of the natural environment if ecosystems become less stable as they become more simple. The consequence may be increasingly unstable populations leading to extinctions, further simplification and even more instability. That there might be a single relationship between such gross variables as stability and complexity is intriguing theoretically, in a field where generalizations are scarce. The evidence on this topic is controversial. Early theoretical studies were logically simplistic, if appealing, in suggesting that complex system were more stable. Later theoretical studies were more sophisticated and suggested exactly the opposite. Field studies are, superficially, ambiguous and contradictory. I shall try here to resolve some of this controversy by pointing to several different definitions of stability, of complexity and of various ecological variables of interest. Several score permutations of these definitions are possible and different ones will yield different results. The earlier theoretical treatments and field studies typically address different combinations from those of later studies; in the few cases where they treat the same combination, there is often good agreement. Most combinations, however, have not been explored and very few have been studied extensively by both theoreticians and field workers.

Early studies¹⁻⁴ argued that increased complexity enhanced ecosystem stability. This seemed so certain that the idea became a central feature of ecology texts. Watt⁵ makes one of his core principles of ecology '... the accumulation of biological diversity ... promotes population stability'. Later studies⁶⁻²¹ typically came to the opposite conclusion. To see why early confidence was misplaced is easy with hindsight. The first of Elton's six arguments was a theoretical one: simple population models are characterized by oscillations; more complex models were expected to fluctuate less. Another theoretical argument, that of MacArthur², was that the more pathways for energy to reach a consumer, the less severe would be the failure of any one pathway. Perhaps because it represents conventional wisdom ('don't put all your eggs in one basket'), the argument was not given a formal mathematical treatment. The conflict between this argument and the diametrically opposite one from later studies is one I shall try to resolve.

One of Elton's lines of evidence involved the increased chance of pest outbreaks in agricultural systems, another the absence of pest outbreaks in tropical (but not temperate) forests and the prevalence of population cycles in the Arctic and, finally, the ease with which species can invade small, remote (and hence species-poor) oceanic islands. Elton's data were few: the absence of pest outbreaks in the tropics was based on a casual conversation with three tropical foresters¹. Furthermore, man's impacts on agricultural systems are many and varied: simplification may not be the only, or even the main cause of their instability.

Simply, the early theoretical arguments and field studies were heterogeneous and 'incomplete'²². Yet this heterogeneity only points to the fact that there are many questions to be answered

in the discussion of stability-complexity relationships. No single question has logical supremacy.

Definitions

Table 1 lists definitions I shall use to build an array of complexity-stability questions. This set of definitions is not exhaustive²³⁻²⁶; I have included those definitions that have been most studied or which promise to be amenable to both theoretical and field studies. One can create 'complexity-stability' questions by taking the possible combinations of measures of complexity, stability and variables of interest. Looking at all the possible combinations suggests some features that should be discussed before answers are sought.

(1) The definitions of stability imply different kinds of comparisons. If a system is not stable (in the strict sense of Table 1), we will probably not observe it except in transition to a new equilibrium²⁷. Rather special dynamics are required to permit

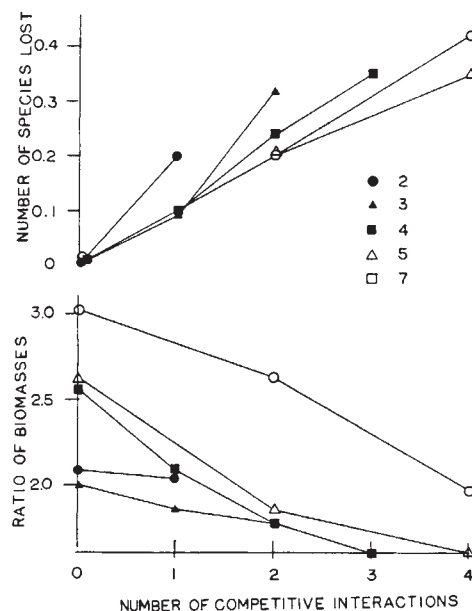


Fig. 1 An illustration of the diametrically opposite effects of increasing complexity on two different measures of stability. Results are from model systems where the single herbivore is removed from a system of possibly competing plants. As the number of competitive interactions between plants increases (abscissa), the system is more likely to lose plant species (upper graph) but change biomass less (lower graph) when the herbivore is removed. In the lower graph, the ratio of biomass plotted is the total biomass of plants without herbivore/total biomass of plants with herbivore. Numbers indicate numbers of plant species in the system.

Table 1 Definitions of variables

Complexity	
Species richness	The no. of species in a system.
Connectance	The no. of actual interspecific interactions divided by the number of possible interspecific interactions.
Interaction strength	The mean magnitude of interspecific interaction: the size of the effect of one species' density on the growth rate of another species.
Evenness	The second moment (variance) of the species abundance distribution measures how uneven are the abundances. (Diversity indices are measures that combine the evenness of the species' abundances (equitability) and species richness. The relative weighting of these two components varies from index to index. One member of this family is the information theoretic index, H .)
Stability	
Stable (units nondimensional and binary: 0 unstable, 1 stable)	A system is deemed stable if and only if the variables all return to the initial equilibrium following their being perturbed from it. A system is locally stable if this return is known to apply only certainly for small perturbations and globally stable if the system returns from all possible perturbations. The set of all values of the variables from which the system returns to a particular equilibrium is known as the domain of attraction.
Resilience (units of time; characteristic return time is time taken for a perturbation to return to $1/e$ (~37%) of initial value.)	How fast the variables return towards their equilibrium following a perturbation. Resilience is not, therefore, defined for unstable systems.
Persistence (units of time)	The time a variable lasts before it is changed to a new value. Turnover is the reciprocal of persistence.
Resistance (units nondimensional and continuous)	The degree to which a variable is changed, following a perturbation.
Variability (the units are those of animals squared per unit area (variance), animals per unit area (s.d.) dimensionless (c.v.))	The variance of population densities over time, or allied measures such as the standard deviation of population densities, or the coefficient of variation (standard deviation divided by the mean density c.v.).
Variables of interest	
Individual species abundances	The densities of all the species in the system.
Species composition	The list of all the species in the system.
Trophic level abundance	The total density (or biomass) of all the species in a particular trophic level (or for comparable definitions, for some other interesting set of species).

species to persist with population densities which do not return to an equilibrium, but instead, cycle indefinitely. Such dynamics are theoretically fascinating but seem to characterize only a few populations²⁸, such as lemmings and lynx. Thus, when we investigate the chances of stability of systems with different complexities we are implicitly comparing existing with hypothetical systems. For the other measures of stability, however, we could ask whether existing systems are more or less resilient, resistant, persistent or variable. (Although if they are not resilient, resistant or persistent enough or too variable, then, indeed, we may not observe them for long.)

(2) There are many kinds of perturbations to natural systems. Detailed answers to specific complexity–stability questions may depend on what is perturbed, when, and by how much. Some perturbations may involve changes in species abundances (like the English winter of 1962–63 which temporarily depressed bird populations²⁹); others may involve the removal of some or all of the species (as in secondary plant succession) and thus involve recoveries of a much longer duration. Consequently, the definitions of resilience, persistence and resistance pose problems of scale when we attempt to measure them in the field³⁰. It is obvious that large perturbations will disappear more slowly than small ones. The time taken for a perturbation to diminish to a given percentage of its initial value, however, may be relatively independent of the size of the perturbation. Such a statistic thus avoids the complication of the initial size of the perturbation, which will depend not only on the size of the disturbance but also on the system's resistance. The notion of persistence is more difficult to deal with. How long a system persists will not only depend on the system, but also on the properties of the disturbances. Defining comparable disturbances for different systems may often be difficult or impossible. But for some cases to be discussed, the differences in persistence clearly can be seen

to depend on the intrinsic differences between systems and not on the disturbances.

It is also obvious that long-lived organisms (say, trees) will be less resilient and more persistent in numbers than short-lived organisms (say, annual plants) on a time scale measured in years³⁰. Why some systems are dominated by organisms with short or long life histories may be the answer to some questions about resilience and resistance. For others, we may wish to scale resilience or persistence further by correcting for the generation times of the species involved.

Perturbations also need to be defined spatially. Disturbing 1 m² is not merely a smaller perturbation than disturbing 1 km²: in the latter, the boundary is smaller relative to the total area, so immigration and emigration are likely to be relatively less important than birth and death processes.

In short, because of the problems of spatial and temporal scales, it may be impossible to compare widely different ecosystems. Yet for similar systems, contrasts of resilience, resistance, persistence and variability should be possible. Indeed, the field studies I shall discuss confirm this.

(3) The definitions of stability are interrelated in complicated ways. Under some circumstances, resistant systems, because they change less under a given disturbance, will appear more persistent and less variable. For some populations, increasing resilience implies decreasing variability²⁹ and, as discussed above, resilience and resistance may or may not be confounded depending on how the former is scaled. I shall not discuss these relationships, in part because Harrison²⁶ has already done so. However, the principal reason is that despite the potential for the reverse, theoretical and even field results fall cleanly into the various definitions of stability; and even if the correlation between any two definitions is absolute, we will retain both when, under different circumstances, each variable becomes an

Table 2 Theoretical and field studies on various combinations of measures of complexity, variables of interest and measures of stability

	THEORETICAL STUDIES		FIELD STUDIES	
	SPECIES RICHNESS	CONNECTANCE AND INTERACTION STRENGTH	SPECIES RICHNESS	CONNECTANCE AND INTERACTION STRENGTH
Individual species abundances	STABLE	Local stability: with more species there is less chance of stability ⁶⁻²¹ Modifications: (1) feasibility ³² (2) structure ^{10,11,31} (3) parameters ^{10,11} (4) donor-control ¹⁰ (5) succession, deletion and evolution ³³⁻³⁶ (6) predator foraging patterns ^{42,43}	1) $C_n \leq \text{constant}$ ⁴⁴⁻⁴⁶ 2) Food webs lack links that are particularly destabilizing ⁴⁷ . 3) Insect systems are more connected than vertebrate dominated ones ⁵⁰	
	RESILIENT	With more trophic levels there is less resilience ^{53,54}	With more species there is less resilience ⁵⁵	
	PERSISTENT	1) Systems with more species are harder to invade ^{36,56} 2) With more species, it is less likely an invader will be a new species	1) Succession: older, systems with more species are more persistent ⁵⁸ 2) Islands: those with fewer species are easier to invade ^{1,59,60}	
Species composition	RESISTANT			
	STABLE	Species deletion: with more species there is less chance of stability (except for donor-control where the reverse is true ^{38,61})	Indirect evidence: most systems are changed considerably by predator removals and are thus not donor-controlled ³⁸	
	RESILIENT			
	PERSISTENT		Geologically, systems with more species persist less ⁶³	
Total Density	RESISTANT			
	RESILIENT	With more connectance there is possibly more resilience ^{64,65}		
	RESISTANT	With more species, there is usually less resistance ⁶⁶		

easier or more appropriate measurement of stability.

(4) The variables of interest are hierarchial in nature. If individual species abundances are maintained, then so are the number of species in the system, species composition and the total density or biomass of any subset of species. Yet species composition may remain the same while the densities of the species change, or vice versa.

(5) Connectance and interaction strength are closely related: their product measures how strongly all the species in the system interact. Theoretically, it is convenient to distinguish species pairs that interact from those that do not. Practically, we might expect a continuous gradation of interaction strengths from the strong to the weak and for these two measures to affect stability in comparable ways.

Table 2 compiles theoretical and field studies on some of the combinations of measures of complexity, variables of interest

and measures of stability. Later, I shall discuss evenness as a measure of complexity and variability as a measure of stability. I shall first summarize what results are available, then conclude by pointing to some questions which appear experimentally and theoretically tractable and yet which do not appear to have been answered.

Individual species abundances

Stability. The majority of theoretical studies have examined local stability of species abundances. Initial results⁶⁻⁹ found that increasing species richness, connectance or interaction strength decrease the chance that randomly assembled communities would be stable. Random assembly is an unreasonable assumption and other studies relaxed the assumptions on the structure and limits of the interaction parameters^{10,11,31,32}. In addition,

models were allowed to develop through the successive additions or deletions of species^{33–36}.

The many subtleties to this work are reviewed by May³⁷, McDonald¹³ and Pimm²⁷ and one feature seems capable of completely reversing the original result. In most models, increasing the density of the predator, decreases the population growth rate of the prey. Alternatively, predators may consume prey that are likely to die or have died from other causes—disease or starvation, for example. For these donor-controlled models, increasing connectance and species richness increases the chances of finding a locally stable model¹⁰. This result matches MacArthur's² idea that complexity buffers the consequences of density variations in one prey species. His argument is, however, incomplete: it fails to consider the variations in densities of species at higher trophic levels. For these, the opposite is true: variations propagate more widely the more connected the system. It is this latter effect that predominates in many models but, by definition, is absent in donor-controlled models. Donor-control dynamics predict that predator removal should have no effect on prey species densities and certainly not on the resultant community's species composition. Usually this is not the case³⁸. With the rejection of donor-control dynamics goes one plausible model where increased stability comes from increased complexity.

In Lotka–Volterra models, resource limitation of species at the base of the food web helps to stabilize the system, and predator effects on prey density (absent in donor-controlled models) are destabilizing. However, the predator interactions, themselves, can be stabilizing. Typical examples of this involve some mechanisms for the prey to escape predation at low densities. Here, predators might not be able physically to attack the prey because the latter are hiding or, perhaps, because low densities cannot be profitably exploited. In these models there can be stable interactions with prey depressed to levels far below those set by resource limitation. Detailed case histories³⁹, general arguments about the structure of herbivore communities⁴⁰, or about herbivores in general⁴¹ all suggest that many species may be limited primarily by predators rather than resources. For such cases Nunney⁴² showed that increasing complexity and species richness could lead to an increased chance of local stability. The generality of Nunney's arguments have been questioned⁴³ but they also require the product of connectance (C) and species richness (n) to be sufficiently large ($Cn > 6$) to reverse the usual decrease in stability. I shall return to this point presently.

Is there any field evidence for these theoretical results? As argued above, the nature of the definition of local stability makes it unlikely (though not impossible) that we will observe anything other than locally stable systems. Many models suggest that, if interaction strength (i) is assumed to be relatively constant, then C should vary with $1/n$ in order to maintain local stability. Data from published food webs^{27,44} or from more detailed studies of lizard communities⁴⁵ show that C does decrease linearly with $1/n$ or nearly so⁴⁶ with $Cn = 3–4$. These values, incidentally, seem too low for Nunney's argument, in the previous paragraph, to hold. Unfortunately, these results say nothing more than that each species interacts with a fixed number of other species as n increases—a result that seems reasonable without recourse to stability explanations⁴⁷. There are, however, certain other patterns of species interactions (particularly those that involve species feeding on more than one trophic level⁴⁸, and the grouping of species interactions within a community⁴⁹) that are particularly destabilizing in models. Natural systems show a statistical scarcity of these patterns^{49,50}. Although interaction strength is hard to measure, it is likely to be less between an insect and its comparably sized insect parasitoid than between a large vertebrate predator and its small prey. Thus, stability should permit insect–parasitoid systems to be substantially more connected than vertebrate-dominated systems⁴⁸. They are⁵⁰. (With the exception just noted, most multi-species models do not consider parasitoid or parasite systems. Yet their distinctive features⁵¹ and ecological importance⁵², plus their greater poten-

tial complexity, suggest that other results from local stability analyses may not be simply transferable to them.)

In sum, most theoretical studies of local stability are best interpreted by saying that the systems we observe should not be too complex. Field evidence suggests this is correct; indeed, patterns of species interactions that are particularly destabilizing in models are also rare in nature.

Resilience. In models, systems with more trophic levels (one way of adding species) are less resilient^{53,54} and, for a given number of trophic levels, stable systems will often^{11,27}, but not always⁴³, be more resilient the more complex they are. Resilience is amenable to field determination²⁹ and unlike stability, permits comparisons of existing natural systems. However, I know of only one such study⁵⁵. It found that plants in a species-poor system recovered more quickly from an unusual drought than those in a nearby species-rich field.

Persistence. How long should species densities continue to return to a given equilibrium and not to some other equilibrium involving the same or some different set of species? One long-term question is whether constantly simple systems persist longer than complex ones (for example, are the species abundances of species-poor desert communities more persistent than those of species-rich tropical forests?). I shall consider this topic below. A second, relatively short-term view, posits changes in both composition and individual abundances, effected, perhaps, by the addition of a species to the system. This presumes that the community has fewer species than the set of those capable of reaching the community (this set is called the species pool). This topic is particularly relevant to islands (either typically oceanic ones or abstract islands of isolated habitat which may lack a full complement of species) or communities which, following a major disturbance, may also lack species.

How hard are communities to invade? Those that are hard to invade will be persistent and vice versa. Models that examine species competing for resources arranged along some gradient⁵⁶ and more general models of food web assembly^{36,57} suggest that communities with more species are harder to invade. In addition, the smaller the fraction of the species pool present in a community, the more likely an invading individual will be of a new species. The more new species that invade per unit time, the more likely it is that one of them will be able to increase and so change the existing system's equilibrium. Simply, both effects mean that the fewer the species in the system, the less persistent that system will be. The effect of increased interaction between species is also to increase persistence^{56,57}.

There are two kinds of field evidence for these effects. First, during succession, species that enter the community later (when there are usually more species) persist longer than those that enter earlier⁵⁸. In part, this must be due to longer-lived organisms arriving later. The question of whether later-arriving organisms persist longer relative to their generation times has not been asked. Of course, that longer-lived organisms arrive later may simply be a reflection of the greater likely persistence of later successional stages. The second evidence avoids these difficulties, for even among species with similar generation times, species are more likely to colonize remote, species-poor islands successfully than species-rich continents^{1,59,60}.

Species composition

Stability. If individual species abundances remain fixed, then so does species composition; thus, much of the previous section applies to this one. It remains to ask, under what conditions does species composition remain unchanged, even though individual abundances change considerably? There are two kinds of studies. The first kind considers the effect of removing species on the composition of the remaining species ('species deletion stability'). Other studies consider whether, following changes in density, species abundances will return to their original equilibrium or move to some new equilibrium involving different abundances but the same composition.

Theoretically, species deletion stability decreases with increasing numbers of species and connectance⁶¹, but it also depends

critically on which species are selected for removal³⁸. If model plants are removed, more species and greater connectance will lead to a lessened chance of losing more species: these results confirm MacArthur's² intuition. For model predators, however, the result is reversed. Of course, donor-controlled systems are not affected by predator removals and, for these, more complex systems will be more species deletion stable³⁸. As noted above, donor-controlled dynamics do not predominate in nature³⁸.

A second problem involves populations which appear to continue at one level for years, then shift to another one and remain there. Lotka-Volterra models do not have this property—more complex dynamics with more parameters are required⁶². In this special sense, systems with greater (dynamic) complexity may permit species composition to remain unchanged when comparable simple models would predict species losses.

Resilience. How fast species composition returns to equilibrium following, say, a major reduction in species, is a question central to the topic of ecological succession. Data are certainly available on how fast succession proceeds. However, I know of no synthesis that relates the rate at which disturbed systems approach their former composition to the various definitions of complexity.

Persistence. I have already noted that species composition should and does last longer when more species are present—at least when viewing the process of succession. But what about the non-successional differences among systems, the species richness of forests versus deserts, for example? In the long term, we might expect development of better prey or predators and changing environmental conditions to cause species losses. From the results on species deletions, we might expect systems with more species to last for shorter periods. The relationship of the persistence of species composition to species richness, therefore, may be dependent on the timescale considered. Boucot⁶³ finds that for marine fossil assemblages 'trophically complex systems... are far more fragile and subject to extinctions' than simpler systems and, hence, tend to last for shorter periods. As E. R. Pianka has observed (personal communication), excepting succession, species compositions of many systems are persistent over at least human life spans.

Total density or biomass

The total density (or biomass) of all or some subset of the species in an ecosystem may correlate far more closely with our notions of what defines an ecosystem than the abundances or identities of particular species. For example, we readily recognize a deciduous forest. But consider, say, 1 km² plots of deciduous forest in eastern North America or western Europe. Within these plots there will be a wide range of tree species, an even greater variation in the abundance of individual tree species, but not, I suspect, a large variance in the total biomass of all tree species.

Resilience. Theoretically, densities of certain sets of species can be more resilient than those of their constituent species and, moreover, this resilience can increase with increasing connectance^{64,65}. I know of no field study that relates biomass resilience during succession to complexity.

Resistance. There are both theoretical^{66,67} and experimental^{55,68,69} studies of the degree of change of biomass and density with the addition or removal of a species or resource. Two of these studies^{66,69} focus on the effects of large mammalian grazers on plant biomass. Models with few plant species but more connectance (interactions between plants) have greater resistance⁶⁶. The former result also holds for very complicated ecosystem models⁶⁷. Greater evenness of prey abundances (a measure of complexity not yet discussed) correlates with greater resistance both theoretically⁶⁶ and in field studies of mammalian grazing⁶⁹.

Other variables

I have not discussed variability as a measure of stability because I believe there are difficulties in testing the various theoretical

ideas. How much populations vary will depend not only on intrinsic factors involving ecosystem complexity⁷⁰⁻⁷², but on the extrinsic nature and frequency of the perturbations. In the species-rich tropics, populations do not vary less than in temperate systems^{22,72,73}. Yet populations do vary more in climatically unpredictable systems than in predictable ones⁷³, suggesting that extrinsic factors may govern variability more than intrinsic ones.

Nor have I discussed pest outbreaks. While it may be that pest species are the most variable⁷⁴, it is more likely that they are simply those species whose densities are high relative to what humans require them to be. Pimental's⁷⁵ finding of lower densities of herbivorous insects with greater species richness might be explained by considerations of equilibrium densities rather than by the definitions of stability used here. The determinants of equilibrium densities⁷⁶ or human requirements⁷⁷ fall outside the scope of this review, but within its scope is the question of what determines how fast an insect population returns to an economically unacceptable equilibrium after being severely reduced by insecticides. I have already considered resilience a measure of stability. In contrast, for pest outbreaks, a resilient population might be considered unstable, while one that recovered slowly might be considered stable. Resilience might indicate stability or instability depending on one's viewpoint⁷⁸. As noted above, with fewer species, resilience tends to increase: whether this is a general explanation of outbreaks is an intriguing possibility, but one clearly based on too few theoretical and field studies.

Conclusions

Theoretically, the more species that are present in a community: (1) the less connected it should be, if it is to be stable, (2) the less resilient will be its populations, (3) the greater will be the change in composition and in biomass when a species is removed, (4) the longer the persistence of species composition in the absence of a species removal. The more connected a community: (1) the fewer species it must have if it is to be stable, (2) the more likely it is to lose other species if one is removed, but (3) the more resilient will be its populations, (4) the more persistent will be its composition and (5) the more resistant will be its biomass if a species is removed. Clearly, different complexity-stability questions yield different answers (Fig. 1). The fact that they do, often explains some of the earlier controversy generated, it is now obvious, by different authors addressing different questions.

Which questions have not been examined? Local stability is a clearly defined concept mathematically and the majority of theoretical studies examine it, alone²⁰. Field studies have not usually addressed local stability because, I suspect, it involves a comparison of existing and hypothetical systems. What field studies have examined are questions that permit comparisons of existing systems and which involve more general views of a system than individual species' abundances. How fast does species composition or total biomass return to equilibrium, how long does composition remain unchanged and how much is composition or total biomass changed following, say, the addition of a species? These questions are as theoretically tractable as those of local stability. Similarly, some accessible theoretical ideas—like resilience—are rarely measured in the field, despite the potential to do so. Put simply, progress in our understanding of the complexity and stability of ecosystems will come from more theoretical and field studies, but is likely to be fastest when these studies consider which questions are both theoretically tractable and experimentally testable: I suggest the many gaps in Table 2 provide a guide to what these questions are.

I thank R. A. Armstrong, J. H. Lawton, R. M. May, S. J. McNaughton and E. R. Pianka for comments on earlier versions of this paper. This research was supported by the NSF's Ecosystems Studies Program under Interagency Agreement DEB 77-25781 with the US Department of Energy under contract W-7405-eng-26 with Union Carbide Corporation: Environmental Sciences, Oak Ridge National Laboratory.

1. Elton, C. S. *The Ecology of Invasions by Animals and Plants* (Chapman & Hall, London, 1958).
2. MacArthur, R. H. *Ecology* **36**, 533–536 (1955).
3. Hutchinson, G. E. *Am. Nat.* **93**, 145–159 (1959).
4. Margalef, R. *Perspectives in Ecological Theory* (University of Chicago Press, 1968).
5. Watt, K. E. F. *Principles of Environmental Science* (McGraw-Hill, New York, 1973).
6. Gardner, M. R. & Ashby, W. R. *Nature* **228**, 784 (1970).
7. May, R. M. *Stability and Complexity in Model Ecosystems* (Princeton University Press, 1973).
8. McMurtrie, A. J. *J. theor. Biol.* **50**, 1–11 (1975).
9. Gilpin, M. E. *Nature* **254**, 137–139 (1975).
10. DeAngelis, D. L. *Ecology* **56**, 238–243 (1975).
11. Pimm, S. L. *Theor. Popul. Biol.* **16**, 144–158 (1979).
12. Siljak, D. *Nature* **249**, 280 (1974).
13. MacDonald, N. *Nature* **275**, 117–118 (1978).
14. Tuljapourkar, S. D. & Semura, J. S. *Nature* **275**, 388–389 (1975).
15. Jeffries, C. *Theor. Popul. Biol.* **7**, 149–166 (1975).
16. May, R. M. *Nature* **238**, 413–414 (1972).
17. Tansky, M. *Mem. Coll. Sci. Kyoto Univ.* **B7**, 87–94 (1978).
18. Webber, M. I. in *Ecological Stability* (eds Usher, M. B. & Williamson, M. W.) 165–178 (Chapman & Hall, London, 1974).
19. Kirkwood, R. S. M. & Lawton, J. H. *J. theor. Biol.* **93**, 225–237 (1981).
20. Goh, B. S. *Math. Biosci.* **40**, 157–166 (1978).
21. Kindlemann, P. & Rejmanek, M. *J. theor. Biol.* **94**, 989–993 (1982).
22. Goodman, D. Q. *Rev. Biol.* **50**, 237–266 (1975).
23. Botkin, D. B. & Sobel, M. J. in *Ecosystem Analysis and Prediction* (ed. Levin, S. A.) 144–150 (Society of Industrial and Applied Mathematics, Philadelphia, 1975).
24. Innis, G. in *Ecosystem Analysis and Prediction* (ed. Levin, S. A.) 131–139 (Society of Industrial and Applied Mathematics, Philadelphia, 1975).
25. Orians, G. H. in *Unifying Concepts in Ecology* (eds van Dobben, W. H. & Lowe-McConnell, R. H.) 139–150 (Junk, The Hague, 1975).
26. Harrison, G. W. *Am. Nat.* **113**, 659–669 (1979).
27. Pimm, S. L. *Food Webs* (Chapman & Hall, London, 1982).
28. Tanner, J. T. *Ecology* **47**, 733–745 (1966).
29. Pimm, S. L. in *Ecological Communities: Conceptual Issues and the Evidence* (eds Strong, D. R. Jr, Simberloff, D. S. & Abele, L. G.) (Princeton University Press, in press).
30. Connell, J. H. & Sousa, W. P. *Am. Nat.* **121**, 787–824 (1983).
31. Lawlor, L. R. *Am. Nat.* **112**, 445–447 (1978).
32. Roberts, A. *Nature* **251**, 607–608 (1974).
33. Tregonning, K. & Roberts, A. *Bull. math. Biol.* **40**, 513–524 (1978).
34. Tregonning, K. & Roberts, A. *Nature* **281**, 563–564 (1979).
35. Roberts, A. & Tregonning, K. *Nature* **288**, 265–266 (1981).
36. Post, W. M. & Pimm, S. L. *Math. Biosci.* **64**, 169–192 (1983).
37. May, R. M. in *Population Dynamics* (eds Anderson, R. M., Turner, B. R. & Taylor, L. R.) 385–407 (Blackwell, Oxford, 1979).
38. Pimm, S. L. *Oikos* **35**, 139–149 (1980).
39. Hassell, M. P. *J. Anim. Ecol.* **49**, 603–628 (1980).
40. Lawton, J. H. & Strong, D. R. Jr *Am. Nat.* **118**, 317–338 (1981).
41. Hairston, N. G., Smith, F. E. & Slobodkin, L. B. *Am. Nat.* **84**, 421–425 (1960).
42. Nunnely, L. *Am. Nat.* **115**, 639–649 (1980).
43. Abrams, P. A. & Taber, D. A. *Am. Nat.* **119**, 240–249 (1982).
44. Rejmanek, M. & Stary, P. *Nature* **280**, 311–313 (1979).
45. Pianka, E. R. in *Ecology and Evolution of Communities* (eds Cody, M. L. & Diamond, J. M.) 291–314 (Harvard University Press, 1975).
46. Yodzis, P. *Nature* **284**, 544–545 (1980).
47. Pimm, S. L. *Nature* **285**, 591 (1980).
48. Pimm, S. L. & Lawton, J. H. *Nature* **268**, 329–332 (1978).
49. Pimm, S. L. & Lawton, J. H. *J. Anim. Ecol.* **49**, 879–898 (1980).
50. Pimm, S. L. *Ecology* **61**, 219–225 (1980).
51. Dobson, A. P. in *Population Biology of Infectious Diseases* (eds Anderson, R. M. & May, R. M.) 1–25 (Springer, Berlin, 1982).
52. Anderson, R. M. *Population Dynamics of Infectious Diseases* (Chapman & Hall, London, 1982).
53. Pimm, S. L. & Lawton, J. H. *Nature* **268**, 329–331 (1977).
54. Vincent, T. L. & Anderson, L. R. *Theor. Popul. Biol.* **15**, 217–231 (1979).
55. Leps, J., Osbornova-Kosinova, J. & Rejmanek, M. *Vegetatio* **50**, 53–63 (1982).
56. May, R. M. & MacArthur, R. H. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1109–1113 (1972).
57. Robinson, J. V. & Valentine, W. D. *J. theor. Biol.* **81**, 91–104 (1979).
58. Shugart, H. H. & Hett, J. M. *Science* **179**, 1279–1281 (1973).
59. Long, J. L. *Introduced Birds of the World* (David & Charles, Newton Abbott, 1981).
60. Moulton, M. P. & Pimm, S. L. *Am. Nat.* **121**, 669–690 (1983).
61. Pimm, S. L. *Oikos* **33**, 351–357 (1979).
62. May, R. M. *Nature* **269**, 471–477 (1977).
63. Boucot, A. J. *J. Paleont.* **57**, 1–30 (1983).
64. Armstrong, R. A. *Am. Nat.* **120**, 391–402 (1982).
65. Armstrong, R. A. *Oak Ridge natn. Lab. Tech. Memo.* (in the press).
66. King, A. W. & Pimm, S. L. *Am. Nat.* **122**, 145–149 (1983).
67. Austin, M. P. & Cook, B. F. *J. theor. Biol.* **45**, 435–458 (1974).
68. Hurd, L. E., Mellinger, M. V., Wolf, L. L. & McNaughton, S. J. *Science* **173**, 1134–1136 (1971).
69. McNaughton, S. J. *Am. Nat.* **111**, 515–525 (1977).
70. DeAngelis, D. L. *Bull. math. Biol.* **37**, 291–299 (1975).
71. Saunders, P. T. & Bazin, M. J. *Nature* **256**, 120–121 (1975).
72. Leigh, E. F. in *Ecology and Evolution of Communities* (eds Cody, M. L. & Diamond, J. M.) 74–80 (Harvard University Press, 1975).
73. Wolda, H. *Am. Nat.* **112**, 1017–1045 (1978).
74. Watt, K. E. F. *Can. Ent.* **97**, 887–895 (1965).
75. Pimentel, D. *Ann. ent. Soc. Am.* **54**, 76–86 (1964).
76. Beddington, J. R., Free, C. A. & Lawton, J. H. *Nature* **273**, 513–519 (1978).
77. Conway, G. in *Theoretical Ecology* (ed. May, R. M.) 356–386 (Blackwell, Oxford, 1981).
78. Turelli, M. *Theor. Popul. Biol.* **13**, 244–267 (1978).

ARTICLES

The Hubble constant as derived from 21 cm linewidths

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The IR 21 cm linewidth–absolute magnitude relation is calibrated by means of local galaxies with known distances, including new distance determinations of M33 and M81. The calibrated relation is applied to 17 Virgo cluster galaxies of type Sab to Sd and yields a cluster modulus of $(m-M)_{\text{Virgo}}^0 = 31.47 \pm 0.34$. Reliable relative distance indicators are used to extend the distance scale to the Coma cluster, for which a distance modulus is found of $(m-M)_{\text{Coma}}^0 = 35.39 \pm 0.35$. The blue 21 cm linewidth–absolute magnitude relation gives slightly larger distances. A combination of the two methods leads to a Hubble constant at the distance of the Coma cluster of $H_0 = 55 \pm 9 \text{ km s}^{-1} \text{ Mpc}^{-1}$. This value agrees with the global value of $H_0 = 50 \pm 7 \text{ km s}^{-1} \text{ Mpc}^{-1}$, giving a Hubble time of $H_0^{-1} = 19.5 \times 10^3 \text{ Myr}$, in agreement with the age of the chemical elements and with the age of our Galaxy, determined from globular clusters.

In a recent discussion of the Tully–Fisher relation¹ it was stated that the extragalactic distance scale and the value of the Hubble constant depend now only on the infall velocity towards Virgo, and on the distances of the galaxies in the neighbourhood of the Milky Way. Because the former is now well determined from an accurate measurement of the relative distance between the Virgo and Coma clusters², and because new fundamental distances to two nearby calibrating galaxies, M33 and M81, have recently become available^{3–5}, it seems justified to look into the consequences of these new developments.

The Tully–Fisher relation, that is the dependence of the luminosity of disk galaxies on their 21 cm linewidth, has been widely used to derive extragalactic distances, either by correlating the absorption-corrected blue *B* magnitude (ref. 6 and refs therein) or the nearly absorption-free IR *H* magnitude (ref. 1 and refs therein) of a disk galaxy with its inclination-corrected 21 cm linewidth Δv_{21} . So far the IR Tully–Fisher relation has consistently led to a surprisingly low Virgo cluster distance of only 16 Mpc, compared with 22 Mpc which follows from other distance indicators, which are insensitive to selection effects and statistical bias^{7,8}. We investigate here this discrepancy, as well as the consequences for the Hubble constant H_0 .

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Data

We restrict the present sample to Sab to Sd spiral galaxies. Very late-type galaxies (Sdm, Sm, Im) are excluded, because knowledge of their intrinsic shape is inadequate to derive reliable inclination angles. Sa galaxies are excluded because there are reasons to doubt that they obey a Tully–Fisher relation; a complete sample of Sa galaxies with $i > 45^\circ$ from the *Revised Shapley–Ames Catalog*⁹ does not reveal a significant dependence of M_B on $\log \Delta v_{21}$. This lack of correlation is possibly related to the fact that Sa galaxies have rotation curves which rise steeply in the central regions. The maximum rotation velocity, $v_{\max}$ —which essentially determines the 21 cm linewidth—measures only the mass of the rotating bulge, which is a fraction of the total mass (and light) of an Sa galaxy¹⁰.

A Tully–Fisher relation has the form

$$M_H = a - b (\log \Delta v_{21} - 2.5) \quad (1)$$

where M_H is the absolute galaxian magnitude in the H band. The constant term a can be determined from nearby calibrator galaxies with known distances (Table 1). The slope b is treated in a first step as a free parameter with values of $b = 10$ –13. The best value of b will be discussed later.

Table 1 contains all spiral galaxies for which fundamental distances are available from Cepheids and/or brightest stars and other distance indicators. The Cepheid distances were not increased by a bulk correction of ~ 0.3 mag, which in the past has seemed to be required by the increased Hyades cluster distance. This open cluster, often used to fix the zero point of the Cepheid period–luminosity function, was originally assigned a distance modulus of $(m - M)^0 = 3.03$, whereas a value of 3.30 seems now much more probable. This change, however, enters with very little weight into the zero point determination of the period–luminosity relation for reasons discussed elsewhere³. Briefly, the zero point from Cepheid radius determinations agrees well with our old zero point without the Hyades increase, and an increase of the Magellanic Cloud moduli by 0.3 mag would conflict with their RR Lyrae star moduli. It seems therefore that our original main-sequence fitting was slightly in error, but that error has just been compensated by the increased Hyades modulus, leaving our first Cepheid zero point^{11,12} nearly unchanged. This now seems to be established via the open cluster NGC6087 which contains the Cepheid S Nor, on the basis of new observations by J. W. Pel (personal communication).

Table 1 Local calibrators

Galaxy	Type	$(m - M)^0$	M_H	$\log \Delta v_{21}$	Weight
NGC224	Sb	24.19*	−23.28	2.75	1
NGC598	Sc	25.23*	−20.89	2.39	1
NGC300	Sc	26.27†	−19.4	2.35	1/2
NGC55	Sc	26.27‡	−20.3	2.18	1/2
NGC247	Sc	26.8§	−19.1	2.35	1/2
NGC253	Sc	27.3	−22.6	2.64	1/2
NGC7793	Sd	27.3	−19.4	2.40	1/2
NGC2403	Sc	27.66¶	−21.21	2.48	1
NGC3031	Sb	28.73#	−24.35	2.71	1
NGC5204	Sd	29.20**	−18.90	2.18	1
NGC5585	Sd	29.20**	−19.05	2.31	1

* Ref. 3.

† Ref. 16: the Cepheids in NGC300 are 7.36 mag fainter than those in the Large Magellanic Cloud, for which an apparent modulus of $(m - M)_{AB} = 18.95$ is assumed.

‡ Assumed to be at the same distance as NGC300; compare ref. 15.

§ From the resolution into stars the galaxy is estimated to be 0.5 mag more distant than NGC300; compare with refs 9, 15.

|| From the resolution into stars the galaxies NGC253 and NGC7793 are estimated to be 1.0 mag more distant than NGC300; compare with refs 9, 15.

¶ Ref. 29; remeasured and slightly adjusted by ref. 5.

Ref. 5.

** Assumed to be at the same distance as M101; compare refs 30, 31.

Two distances in Table 1 need special comment: the distances to M33 and to M81. (1) The distance of M33 (NGC598) has only now been firmly based on Cepheids^{4,5}. Its true distance modulus of $(m - M)^0 = 25.33$ (1.1 Mpc) makes it 1.6 times more distant than M31, provided the distance of the latter galaxy is correct. It has been suggested on the basis of IR photometry of the Cepheids in M31, that the true distance modulus of this galaxy is $(m - M)^0 = 24.6$ (ref. 13), which would reduce the distance ratio of M33 and M31 to 1.3. The increased distance to M31 has conservatively not yet been considered here. (2) Lacking a direct distance determination to M81, it has previously been assumed that M81 lies at the same distance as NGC2403. However, recent measurements of the Cepheids, of the colour-magnitude diagram of the brightest blue and red stars, and of the novae in M81 (ref. 5) leads to an apparent blue modulus of $(m - M)_{AB}^{M81} = 28.8$ which is 1.0 mag larger than adopted previously, based as it was then on the modulus of NGC2403. We should also point out that the distance to NGC2403 adopted in Table 1 may again be conservative, because IR photometry of the Cepheids in that galaxy suggests a true distance modulus of $(m - M)^0 = 28.15$ (ref. 14).

As in the case of the M81 group, it appears now as an oversimplification to assume the ‘members’ of the South Polar Group to lie at a common distance^{9,15}. In the latter group a reliable Cepheid distance is only available for NGC300¹⁶. NGC55 seems to lie at approximately the same distance, judging from its resolution into stars. Unfortunately no H magnitudes are available for these two galaxies; the values in Table 1 were calculated on the assumption that the intrinsic colour of an Sc galaxy is $(B - H) = 1.4$. NGC253 and NGC7793 are judged to be 1 mag more distant on the basis of their resolution into stars, and the same criterion suggests that NGC247 lies between the pairs NGC300/55 and NGC253/7793. These distances are quite tentative, and they are given only half weight in Table 1. NGC300 and NGC55 are also given half weight because of their somewhat uncertain H magnitudes. The IR $H_{-0.5}$ magnitudes, corrected for absorption, were taken from refs 17 and 19. The 21 cm linewidths were averaged from refs 6, 17 and 19. The Tully–Fisher relation of the calibrators is plotted in Fig. 1.

Values of the constant term a of equation (1), as determined by the calibrator galaxies, are listed in Table 2 for different values of the slope b . The first set of solutions for a is based on the six calibrator galaxies with full weight in Table 1; the second set of solutions uses all 11 calibrators with the appropriate weights given in Table 1. Comparison of the two sets shows that the value of a is quite insensitive to whether or not the traditional South Polar Group is excluded. For the following discussion the solution adopted is based on only six calibrators.

Table 3 lists all Shapley–Ames galaxies within 6° of the Virgo cluster centre⁹ with known IR magnitudes $J_{-0.5}^c$ (refs 17, 18) and 21 cm linewidths^{6,18}. Two galaxies, NGC4206 and NGC4498, for which the necessary data are available, were added. The sample is the same as that considered in refs 1 and 18, with the exception that Sa and very late-type galaxies are excluded here. The Tully–Fisher relation of the Virgo galaxies is shown in Fig. 1.

Distance of the Virgo cluster

By means of equation (1) and using the appropriate values of a from Table 2, the individual distance moduli of the Virgo

Table 2 The zero point of the IR Tully–Fisher relation and the distance modulus of the Virgo cluster

b	10.0	11.0	12.0	13.0
a	−21.58	−21.61	−21.64	−21.67
(6 calibrators)	± 0.26	± 0.29	± 0.34	± 0.41
a	−21.50	−21.56	−21.61	−21.67
(11 calibrators)	± 0.28	± 0.30	± 0.33	± 0.37
$(m - M)_{\text{Virgo}}^0$	31.34	31.43	31.52	31.60
(6 calibrators)	± 0.12	± 0.13	± 0.15	± 0.17

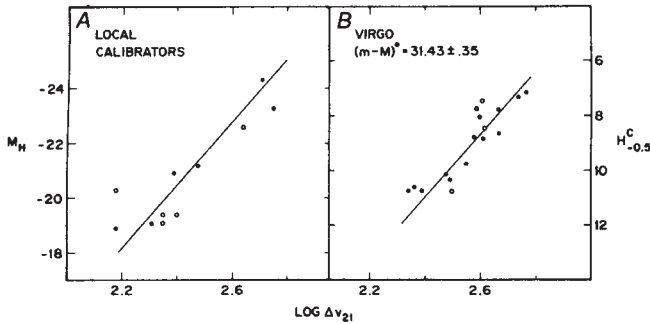


Fig. 1 The IR Tully-Fisher relation. A, The local calibrators with absolute IR magnitudes M_H and 21 cm linewidths from Table 1. B, Virgo cluster galaxies with apparent IR magnitudes $H_{-0.5}^c$ and 21 cm linewidths from Table 3. The open symbols denote low-weight galaxies. The lines in the two diagrams have the adopted slope $b = 11.5$.

galaxies in Table 3 can be calculated. The resulting mean distance moduli for different adopted values of b are listed in Table 2, as well as the mean standard deviation of that mean. An important conclusion is that the Virgo modulus depends on the value of b , varying from $(m-M)_{\text{Virgo}}^0 = 31.34$ for $b = 10$ to $(m-M)_{\text{Virgo}}^0 = 31.60$ for $b = 13$. The slope dependence becomes even stronger if the traditional South Polar Group is included among the calibrators. Following ref. 18 the interacting galaxy NGC4438 and the deviating galaxy NGC4569 are excluded here, and NGC4519 and NGC4535 with uncertain inclinations are given half weight. In view of the slope dependence it is important to determine the best value of the slope b . The six calibrating galaxies in Table 1 give very uncertain values of $b = 9.4$ or $b = 10.0$, depending on whether $\log \Delta v_{21}$ or M_H are treated as the independent variable. In the case of the Virgo galaxies the errors should lie mainly in the Δv_{21} values; hence treating M_H as an independent variable yields $b = 10.5$; this value is again quite uncertain and is sensitive to the weight given to individual galaxies. We therefore adopt the value of $b = 11.5$, which was derived for 300 field galaxies of different types, making allowance for a Virgo-centric infall model¹. With this slope, the r.m.s. scatter of the present Virgo sample becomes $\sigma = 0.53$ mag.

From interpolation in Table 2 one finds for $b = 11.5$ a Virgo modulus of

$$(m-M)_{\text{Virgo}}^0 = 31.47 \pm 0.34 \quad (2)$$

corresponding to a distance of 19.7 ± 3.1 Mpc. Here the mean standard deviation includes the uncertainty of a , but it does not include a remaining systematic error of ± 0.15 mag due to the uncertainty of the slope b .

Table 3 Virgo cluster galaxies

Galaxy	Type	$H_{-0.5}^c$	$\log \Delta v_{21}$
NGC4178	SBC	10.14	2.48
NGC4192	Sb:	7.77	2.67
NGC4206	Sc:	10.34	2.49
NGC4216	Sb	7.33	2.74
NGC4294	SBC	10.77	2.39
NGC4380	Sab	9.77	2.55
NGC4388	Sab	8.86	2.61
NGC4438	Sb (tides)	7.75	2.59
NGC4450	Sab pec	8.05	2.60
NGC4498	SBC	10.76	2.34
NGC4501	Sbc	7.14	2.77
NGC4519	SBC	10.75	2.50
NGC4522	Sc/Sb:	10.60	2.36
NGC4535	SBC	8.45	2.62
NGC4569	Sab	7.45	2.61
NGC4651	Sc	8.65	2.67
NGC4654	SBC	8.79	2.58

Table 4 The relative distance between the Coma and Virgo cluster

Brightest cluster members	3.37 ± 0.40	ref. 32
Ten brightest cluster members	4.22 ± 0.28	ref. 33
($u-V$) colours of E galaxies	3.89 ± 0.20	ref. 34
Supernovae of type I	4.22 ± 0.33	ref. 35
Velocity dispersion of E galaxies	3.75 ± 0.18	ref. 2
Mg_2 index of E galaxies	4.00 ± 0.18	ref. 2
$(m-M)_{\text{Coma-Virgo}}^0$	3.92 ± 0.09	

Value of H_0

The distance of the Virgo cluster combined with its mean recession velocity leads to an unreliable value of the Hubble constant, because the observed recession velocity is affected by streaming motions within the Virgo complex and possibly, although to a lesser extent, by the random peculiar motion of the Local Group. Present determinations of the streaming velocities show them to be of the order of 20% of the Virgo cluster velocity (refs 7, 20 and refs therein).

At the distance of the Coma cluster with a mean recession velocity of almost $7,000 \text{ km s}^{-1}$ any non-Hubble velocity component can hardly amount to as much as 10%, because it is known from the cosmic microwave background that the largest peculiar motion we partake of is $\sim 600 \text{ km s}^{-1}$.

Fortunately the relative distance between the Virgo cluster and the Coma cluster is now determined with exceptional precision from different (relative) distance indicators. The available determinations are listed in Table 4 and yield a mean modulus difference of $\Delta(m-M)_{\text{Coma-Virgo}} = 3.92 \pm 0.09$. The IR colour-luminosity relation of E galaxies, which give decreasing distance differences with increasing wavelengths²¹, have not been considered here, because these colours must either mean that the E galaxies in Virgo and Coma have different IR properties, or they reflect remaining observational difficulties. Indeed, the IR colours are not true simultaneous colour observations, but are the difference of magnitude observations taken by different observers at different telescopes and reduced to a common blue isophote.

The Virgo cluster distance in the previous section combined with the modulus difference from Table 4 gives a Coma modulus of

$$(m-M)_{\text{Coma}} = 35.39 \pm 0.35 \quad (3)$$

corresponding to a distance of $r = 120 \pm 20$ Mpc. The mean Coma cluster velocity, corrected by $300 \sin l \cos b \text{ km s}^{-1}$, is $v_0 = 6,952 \pm 61 \text{ km s}^{-1}$ (ref. 22) or with our preferred correction to the centroid of the Local Group²³, $6,913 \pm 61 \text{ km s}^{-1}$. From this follows

$$H_0 = \frac{v_0}{r} = 58 \pm 10 \text{ km s}^{-1} \text{ Mpc}^{-1} \quad (4)$$

Discussion

We have followed the prescriptions given in refs 1 and 18 for the application of the IR magnitude-21 cm linewidth relation, and have shown that the value of the Virgo cluster modulus via the Tully-Fisher route does depend on the adopted slope b . Using the Aaronson-Mould best value of b , we obtain a modulus of 31.47 ± 0.34 . Although we believe there are difficulties in the Tully-Fisher method when it is pushed to obtain $\pm 20\%$ accuracies, the purpose of this article is to point out that using the prescriptions advocated in ref. 1, we nearly reproduce the preferred value of $m-M = 31.7$ obtained by other methods⁷. The modulus increase over the Aaronson-Mould value of 31.08 ± 0.11 is due almost entirely to two causes: (1) The new fundamental distances of M33 and M81 have changed the constant term a in equation (1) from $a = -21.39 \pm 0.11$ (ref. 1) to $\sim -21.6 \pm 0.3$ (depending on the adopted slope). The change is not larger, because we have not applied the bulk correction to the calibrating galaxies for the increased Hyades modulus. (2) Aaronson and Mould¹ have adopted a slope of $b = 10$, although

their preferred solution gives a slope of $b = 11.54$. They argue that the choice of the slope has negligible effect on the distance modulus, but as shown in Table 2 their steeper slope makes a difference of $\Delta(m - M) = +0.13$. The sensitivity of the solution to the slope has also been emphasized by Richter and Huchtmeier⁶. (Aaronson and Mould¹ have not included in the quoted error their estimated zero point error of 0.2 mag; with this additional error included our error estimates do not deviate significantly.)

In an investigation of the slope of the blue Tully-Fisher relation, Richter and Huchtmeier⁶ have found a slope of $b = 7.17$ and from a large sample of 66 Virgo galaxies a Virgo cluster modulus of $(m - M)_{\text{Virgo}} = 31.82 \pm 0.14$. Adopting their slope, using the local calibrators as shown in Table 1, and including only the 48 Virgo galaxies of type Sab to Sd of their sample, we find $(m - M)_{\text{Virgo}} = 31.66 \pm 0.26$ (the error here includes the error of the constant term $a = -19.82 \pm 0.23$).

It is reassuring for the 21 cm linewidth method, that it yields Virgo cluster distances for IR and blue magnitudes that are almost the same. The mean value of $(m - M)_{\text{Virgo}} = 31.57 \pm 0.25$ agrees well with our independently determined value $(m - M)_{\text{Virgo}} = 31.67 \pm 0.26$ (ref. 7), but we emphasize that the 21 cm method should be viewed with caution until it is better understood why the Hubble-type dependence has not yet been found in the radio data, but is clearly present in the optical results¹⁰.

If one carries the distance scale beyond the Virgo cluster out to the Coma cluster by means of relative distance indicators

given in Table 4, one obtains a value of the Hubble constant which must essentially be free of all irregularities of the expansion field and thus must be very close to the global value of H_0 . Adopting a Virgo modulus based on the IR Tully-Fisher relation leads then to $H_0 = 58 \pm 10$, or $H_0 = 55 \pm 9$ on the basis of the IR and blue Virgo modulus. Both solutions lie well within the error range of the preferred value of $H_0 = 50 \pm 7$ (ref. 7).

There are remaining problems with the Tully-Fisher relation. In addition to the problem of the dependence on galactic type previously mentioned, there is (1) the possibility that the relation is not the same for field and cluster galaxies^{24,25}, and (2) the problem that the true intrinsic dispersion of the relation, if large, discriminates against intrinsically faint galaxies of a given linewidth and leads to an underestimate of the distances. It is therefore a matter of personal judgement how much weight one wants to attribute to the method. It is in any case, however, satisfactory to see that the 21 cm linewidth method, as it begins to mature, leads to results which are now consistent with the established distance scale.

This agreement is particularly satisfactory because it reinforces the time-scale agreement between the Hubble time of H_0^{-1} (50) $= 19.5 \times 10^3$ Myr the age of the chemical elements at $\sim 20 \times 10^3$ Myr (ref. 26), and the age of the globular clusters in our Galaxy at $\sim 18 \times 10^3$ Myr (refs 27, 28).

G.A.T. is a visiting associate of Mount Wilson and Las Campanas Observatories, Carnegie Institution, and acknowledges support from the Swiss NSI.

Received 24 June; accepted 8 November 1983.

1. Aaronson, M. & Mould, J. *Astrophys. J.* **265**, 1-17 (1983).
2. Dressler, A. Preprint, Mount Wilson and Las Campanas Observatories (1983).
3. Sandage, A. *Astr. J.* **88**, 1108-1125 (1983).
4. Sandage, A. & Carlson, G. *Astrophys. J. Lett.* **267**, L25-28 (1983).
5. Sandage, A. *Astr. J.* (in the press).
6. Richter, O.-G. & Huchtmeier, W. K. *Astr. Astrophys.* (in the press).
7. Sandage, A. & Tammann, G. A. *Astrophysical Cosmology* (eds Brück, H. A., Coyne, G. V. & Longair, M. S.) 23-81 (Pontificia Academia Scientiarum, 1982); *Astrophys. J.* **256**, 339-345 (1982).
8. Tammann, G. A. & Sandage, A. *Highlights Astr.* **6**, 301-313 (1983).
9. Sandage, A. & Tammann, G. A. *A Revised Shapley-Ames Catalog of Bright Galaxies*, 6 (Carnegie Institution, Washington, 1981).
10. Rubin, V. C. *IAU Symp. No. 100*, 3-8 (1983).
11. Sandage, A. & Tammann, G. A. *Astrophys. J.* **151**, 531-545 (1968).
12. Sandage, A. & Tammann, G. A. *Astrophys. J.* **157**, 683-708 (1969).
13. Madore, B. F. Talk at IAU General Assembly, Patras (1982).
14. McAlary, C. W. & Madore, B. F. Preprint, Steward Observatory, Tucson (1983).
15. Graham, J. A. *Astrophys. J.* **252**, 474-480 (1982).
16. Graham, J. A. *Highlights Astr.* **6**, 209-216 (1983).

17. Aaronson, M., et al. *Astrophys. J. Suppl.* **50**, 241-262 (1982).
18. Mould, J., Aaronson, M. & Huchra, J. *Astrophys. J.* **238**, 458-470 (1980).
19. Aaronson, M., Mould, J. & Huchra, J. *Astrophys. J.* **237**, 655-665 (1980).
20. Yahil, A., Sandage, A. & Tammann, G. A. *Physical Cosmology* (eds Balian, R., Audouze, J. & Schramm, D. N.) 158 (North Holland, Amsterdam, 1980).
21. Aaronson, M., Persson, S. E. & Frogel, J. A. *Astrophys. J.* **245**, 18-24 (1981).
22. Tift, W. G. & Gregory, S. A. *Astrophys. J.* **205**, 696-708 (1976).
23. Yahil, A., Sandage, A. & Tammann, G. A. *Astrophys. J.* **217**, 903-915 (1977).
24. Kraan-Korteweg, R. C. *Astr. Astrophys.* **125**, 109-111.
25. Giovannelli, R. C. *IAU Symp. No. 104*, 273-278 (1983).
26. Thielemann, F.-K., Metzinger, J. & Klapdor, H. V. *Z. Phys. A309*, 301-317 (1983).
27. Sandage, A. *Astrophys. J.* **252**, 553-573 (1982).
28. VandenBerg, D. A. *Astrophys. J. Suppl.* **51**, 29-65 (1983).
29. Tammann, G. A. & Sandage, A. *Astrophys. J.* **151**, 825-860 (1968).
30. Sandage, A. & Tammann, G. A. *Astrophys. J.* **194**, 223-243 (1974).
31. Sandage, A. *Astr. J.* **88**, 1569-1578 (1983).
32. Sandage, A. & Hardy, E. *Astrophys. J.* **183**, 743-757 (1973).
33. Weedman, D. W. *Astrophys.* **203**, 6-13 (1976).
34. Visvanathan, N. & Sandage, A. *Astrophys. J.* **216**, 214-226 (1977).
35. Tammann, G. A. *Supernovae: A Survey of Current Research* (eds Rees, M. J. & Stoneham, R. J.) 371-403 (1982).

A helix stop signal in the isolated S-peptide of ribonuclease A

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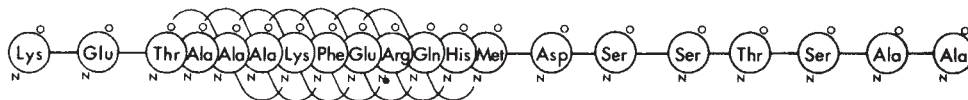
The isolated S-peptide (residues 1-20 of ribonuclease A) is known to show partial α -helix formation in aqueous solutions at low temperatures. We show here that the helix is limited to certain residues, including ones that are helical in the intact protein, and that a functional helix termination signal exists in the isolated peptide.

THE α -helix is the most abundant unit of secondary structure in proteins. Most α -helices are quite short: the average length of α -helices in proteins is about 11 residues¹. In contrast, the average length of α -helices is much longer in synthetic polypeptides (about 100 residues at the transition midpoint)², and therefore, α -helix termination signals exist in proteins. The nature of these helix termination signals is of fundamental interest with respect both to the mechanism of protein folding and the prediction of folding from amino acid sequence. An important question concerning the mechanism of protein folding is whether the helix start and stop signals function at early stages in folding when the α -helices and β -sheets are first formed, or

whether tertiary interactions are required to fix the ends of helices.

The X-ray structures of RNase A³ and of RNase S⁴ show that RNase contains three short helices. [RNase S is an enzymatically active derivative of RNase A cleaved at the peptide bond between residues 20 and 21; the proteolysis products can be separated and are referred to as the S-peptide (residues 1-20) and the S-protein (residues 21-124)⁵.] The structure of interest here is the α -helix formed by residues 3-13 (counting residues containing at least one α -helical H-bond). The crystal structure shows that the amide proton of Asp 14 (the next H-bond donor at the C-terminal end of the 3-13 helix) is H-bonded to the

Fig. 1 Amino acid sequence of S-peptide (1–20), showing the α -helix formed by these residues in native RNase A³ and RNase S⁴. S-peptide (1–20) was prepared and purified for us by V. MacCosham. RNase A (Sigma Type 1-AS, lot no. 81F-8015) was purified¹⁸ and then digested with Subtilisin Carlsberg (Sigma Type VIII, lot no. 48C-0164)¹⁹. S-peptide was purified by ion exchange chromatography (SP-Sephadex C25 in 0.01 M HCl; 0.05–1.0 M NaCl gradient) and by gel filtration (Sephadex G-25 fine in 0.01 M HCl). Two peptides were separated using the purification procedure above, and amino acid analysis (performed at the Department of Biochemistry and Biophysics, University of California, Davis) indicated that the peptides were S-peptide (1–19) and S-peptide (1–20) (see ref. 20). All experiments reported here were made using purified S-peptide (1–20).



carbonyl oxygen of Val 47 in a tertiary interaction (contrast Fig. 4f of ref. 6). Thus, this tertiary interaction holds residue 14 in a non-helical conformation. One would like to know whether this tertiary interaction is required for the localization of the 3–13 helix, or if a functional helix termination signal exists in the absence of tertiary interactions (for example in an early protein folding intermediate).

One direct approach for investigating the nature of helix termination signals is to study the folding of protein fragments. Until recently, however, this has not been possible because even large fragments of single-domain proteins have been found to be unfolded in aqueous solutions^{7,8}. Moreover, 'host-guest' data for α -helix formation in water by synthetic polypeptides⁹ indicate that short α -helices (<20 residues) are always unstable in water, regardless of the amino acid composition. These data have led to the conclusion that secondary structure in protein fragments is not stable in the absence of stabilizing tertiary structure interactions.

Nevertheless, both the C-peptide of RNase A (residues 1–13, obtained by cyanogen bromide cleavage at Met-13, yielding HSer-13 lactone as the C-terminal residue), and the S-peptide (residues 1–20) show partial helix formation in aqueous solutions as judged by circular dichroism (CD) spectra and by ¹H nuclear magnetic resonance (NMR) studies^{10–13}. Some characteristics of the helix formed by the C-peptide are as follows. (1) Up to ~30% helix content is observed in water near 0 °C¹¹, which is 1,000 times greater than the helix content predicted using data from host-guest studies. (2) Helix formation is strongly dependent on temperature, and C-peptide is essentially unfolded in aqueous solutions at 25 °C^{10,11}. (3) Equilibrium sedimentation measurements show that C-peptide is monomeric at concentrations up to 2 mg ml⁻¹ in conditions in which helix formation occurs (1 °C, 0.1 M ionic strength)¹⁰. (4) The pH dependence of helix stability follows a bell-shaped curve¹¹, with maximum helix formation occurring near pH 5. A possible explanation for this pH dependence has been given¹¹: a salt bridge between the ionized sidechains of Glu 9⁻ and His 12⁺ could stabilize the helix.

Here we use S-peptide (1–20), which contains the first 20 amino acids of RNase A, to ask whether the α -helix formed by the isolated peptide stops near residue 13 (the last residue of this helix in native RNase A or RNase S). Five residues with low helical propensity occur in a stretch next to the 3–13 helix (residues 14–18, see Fig. 1). Although studies of helix formation in random copolymers allow some amino acid residues to be classified as 'helix formers' and others as 'helix breakers', the reported stability constant (*s*) values for helix growth span a very narrow range (*s* = 0.5–1.3 for all residues studied)⁹. The 'cooperative length' of an α -helix is about 100 residues² = $1/\sigma^{1/2}$, where σ is the helix nucleation constant; $\sigma \sim 10^{-4}$ for all residues studied⁹. Predicted distributions of helix length for short peptides (including end effects) containing a single residue type are discussed by Schellman¹⁴. For a 20 residue peptide, these results show that a significant fraction of the α -helices formed will include all 20 residues, in the absence of helix termination signals.

To determine whether the α -helix formed by S-peptide (1–20) is localized, we have followed the ¹H-NMR chemical shifts of various sidechain groups as the helix is unfolded. The helices formed by S-peptide (1–20) and C-peptide lactone can be unfolded by increasing temperature^{11,13}, GuHCl¹², or urea, as we report here. Since the stability of the S-peptide (1–20) helix is pH dependent¹³, we have also monitored the ¹H-NMR chemical shifts of sidechain groups as the helix content is changed by varying pH. Of particular interest are those amino acid residue types that are present both within and outside the native 3–13 α -helix (Fig. 1). These include the alanine residues (Ala 4, 5, and 6 are in the helix of native RNase A, whereas Ala 19 and 20 are not), and the threonine residues (Thr 3 is in the native helix and Thr 17 is not). The β -CH₃ group of alanine is physically close to the α -helix backbone, and the alanine β -CH₃ resonance is expected to shift with helix formation, as has already been observed for Ala 4, 5, and 6 in the C-peptide lactone helix¹². The γ -CH₃ resonance of Thr 3 also shifts as the C-peptide lactone helix is formed¹¹, and here we ask whether the Thr 17 (γ -CH₃) resonance shows a comparable helix shift.

Table 1 Comparison between NMR helix shifts and CD changes in S-peptide (1–20) and C-peptide lactone (1–13).

NMR resonance	Peptide	Helix shift $\Delta\delta$ (p.p.m.)*	Molar peptide CD change $\Delta[\theta]_{222}$ (deg cm ² dmol ⁻¹)†	Normalized helix shift $\Delta\delta/\Delta[\theta]_{222}$	Ratio (<i>R</i>) of normalized helix shift S-Peptide (1–20): C-Peptide lactone‡
Thr 3 (γ CH ₃)	S-peptide (1–20)	0.071	4.30×10^4	1.65×10^{-6}	1.07
Thr 3 (γ CH ₃)	C-peptide (lactone)	0.103	6.69×10^4	1.54×10^{-6}	
Phe 8 (ϕ H)§	S-peptide (1–20)	–0.051	4.30×10^4	-1.19×10^{-6}	1.04
Phe 8 (ϕ H)	C-peptide (lactone)	–0.076	6.69×10^4	-1.14×10^{-6}	

* Difference in chemical shift between 4 °C and 47 °C, at the optimum pH for helix formation. S-peptide (1–20) data are for pH 3.8. Data for C-peptide lactone (pH 5.0) are from ref. 11. Temperature dependence of chemical shift, in absence of helix formation, not taken into account.

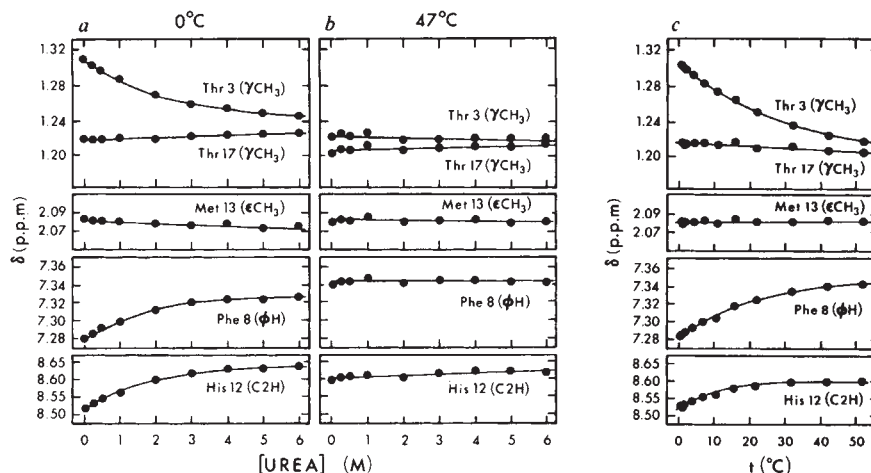
† Difference in $[\theta]_{222}$ per mole peptide between 3 °C and 40 °C (pH 3.8) for S-peptide (1–20)¹³ and between 3 °C and 45 °C (pH 5.0) for C-peptide lactone¹¹.

‡ Predicted value for *R* is 1.0 if the helix is localized to the same residues in both peptides and if the helix shifts are the same for both peptides at 100% helix formation. If the helices propagate to the ends of both peptides, then *R* is predicted to be ≤ 0.63 ($\frac{1}{2}$), corresponding to the ratio of the number of peptide carbonyl groups. Due to end effects on the apparent molar ellipticity of an α -helix (see ref. 24 and refs therein), *R* will be < 0.63 if the helices include all residues of both peptides.

§ There are six visible resonance lines for Phe 8, and only three of them shift significantly with helix formation¹¹. Values are reported for the middle resonance of these three.

Fig. 2 *a*, Urea dependence of ^1H -NMR chemical shift for some side-chain resonances in S-peptide (1–20): 0 °C, pH 3.8, 0.1 M NaCl, D_2O . *b*, Urea dependence: 47 °C, pH 3.8, 0.1 M NaCl, D_2O . *c*, Temperature dependence of ^1H -NMR chemical shift for some side-chain resonances in S-peptide (1–20): pH 3.8, 0.1 M NaCl, D_2O . The average chemical shift for the threonine $\gamma\text{-CH}_3$ doublets are shown. There are six visible resonance lines for Phe 8 (ϕH), and only three of them shift significantly with helix formation. The middle resonance of these three is shown.

Methods: All samples contained 0.1 M NaCl in D_2O . pH was adjusted with DCl or NaOD, and pH values refer to meter readings without isotope corrections. Urea and GuHCl were Schwarz-Mann ultrapure grade. Urea- d_4 and GuDCl- d_6 were prepared by repeated lyophilization from D_2O (Liquid Carbonic, 99.7% C). All other chemicals were reagent grade. Fourier-transform proton NMR spectra were recorded on a modified Bruker 360 MHz instrument at the Stanford Magnetic Resonance Facility. All chemical shifts are reported relative to the internal standard, TSP. Corrections were made for the pH dependence of the TSP signal²¹. Resolution-enhanced NMR spectra were obtained by multiplication of the free-induction decay with either a double exponential²² or a sine bell function²³. Damping of the free-induction decay in the presence of urea was observed (contrast ref. 20), but did not affect the spectra of the peptide. Resonance assignments have been or will be given elsewhere²⁰ (A. Bierzynski *et al.* manuscript in preparation). The Thr 17 ($\gamma\text{-CH}_3$) resonance was assigned by comparing the spectra of S-peptide (1–15) and S-peptide (1–20) in the unfolded state (5.0 M GuDCl, 0° or 47 °C).



Side-chain resonances

The optimum conditions for α -helix formation in aqueous solutions of S-peptide (1–20), as judged by CD, are pH 3.8, 0 °C¹³. As urea is added in these conditions, some side-chain resonances show chemical shift changes while others do not (Fig. 2*a*). The side-chain resonances of Thr 3, Phe 8 and His 12 show changes in chemical shift as the helix is unfolded with urea. However, when the α -helix is already thermally unfolded at 47 °C, urea does not affect the chemical shifts of these resonances (Fig. 2*b*).

In contrast to Thr 3, the side-chain resonance of Thr 17 does not change as the helix is unfolded (Fig. 2). Thus, Thr 17 is probably not in the α -helix formed by the isolated S-peptide (1–20). End effects (helix fraying) are not sufficient to explain the difference observed between Thr 3 and Thr 17, because Thr 3 is the third residue from the amino-terminus and Thr 17 is the fourth residue from the carboxyl-terminus.

The S- CH_3 resonance of Met 13 does not shift as urea is added (Fig. 2). However, the lack of a helix shift for Met 13 is difficult to interpret for two related reasons. (1) The S- CH_3 sidechain group of methionine is four bonds removed from the peptide backbone, and it may be possible for the helix to form without perturbing the magnetic environment of the S- CH_3 protons. (2) We do not have a positive control demonstrating a helix shift for a methionine S- CH_3 resonance, whereas with other resonances (for example threonine $\gamma\text{-CH}_3$), a positive control exists. It is therefore not possible to tell whether Met 13 is contained in the helix formed by isolated S-peptide (1–20). In native RNase A, Met 13 is the last residue in the α -helix. His 12 (ring C2H resonance) does show a helix shift (Fig. 2). The $\beta\text{-CH}_3$ resonances of Ala 4, 5, 6, 19, and 20 are not well separated in spectra taken in urea at high temperatures, or at high urea concentrations, 0 °C, and are therefore not depicted in Fig. 2.

The α -helices formed by both C-peptide lactone and S-peptide (1–20) unfold with increasing temperature^{11,13}. Figure 2*c* depicts the temperature dependences of the chemical shifts for the resonances shown previously, at pH 3.8. The residues that show a helix shift for urea-induced unfolding (Fig. 2*a*) also show a temperature-induced helix shift (Fig. 2*c*). In particular, the Thr 17 resonance, which does not show a urea-induced helix shift (Fig. 2*a*), also does not show a temperature-induced shift (Fig. 2*c*), supporting the suggestion that the S-peptide (1–20) helix is localized.

Guanidine dethiochloride (GuDCl) is known to affect the chemical shifts of many side-chain resonances, and these changes

are often not attributable to the unfolding of structure. Moreover, the effects of GuDCl on chemical shifts are dependent on neighbouring groups, suggesting that the effects are related to the binding of GuDCl to the peptide¹². Our GuDCl titrations of S-peptide (1–20) at 37 °C, where the helix is thermally unfolded, also show GuDCl-induced changes in chemical shift (Fig. 3*b* and 4*b*) and some of these resonances continue to change between 3–5 M GuDCl, where S-peptide (1–20) should be in a 'random coil' conformation.

In addition, GuDCl appears to cause a 'salt effect' on the chemical shifts between 0 and 1 M GuDCl that does not appear to be related to helix structure for the following reasons. (1) The effect is observed even at 37 °C, where the helix is thermally unfolded (Fig. 3*b* and Fig. 4*b*). (2) Essentially all resonances show the salt-effect. These GuDCl-induced chemical shifts may be caused in part by effects of GuDCl on the internal standard (TSP). Both GuDCl and TSP are charged, and we have observed NaCl-dependent shifts of the TSP resonance relative to acetone or dioxane.

When the GuDCl titrations are performed at 0 °C (favouring helix formation), some resonances show changes in chemical shift that evidently are caused by helix unfolding (Fig. 3*a* and Fig. 4*a*). To determine the changes in chemical shift caused by helix unfolding, we have subtracted the GuDCl dependence of the chemical shifts at 37 °C, in the absence of helix formation, from the dependence at 0 °C where the helix is populated. The temperature difference curves for the GuDCl titrations are depicted in Fig. 3*c*. As was found with urea and temperature, the side-chain resonances of Thr 3, Phe 8, and His 12 show helix shifts with GuDCl, whereas those of Met 13 and Thr 17 do not (Fig. 3*c*).

An advantage of using GuDCl is that resonances are spread out which arise in similar regions of the NMR spectrum. In particular, the five alanine $\beta\text{-CH}_3$ groups (Ala 4, 5, 6, 19, and 20) give rise to ten resonance lines in the NMR spectrum of S-peptide (1–20). There is considerable overlap of these resonances in the spectrum, under conditions in which the helix is unfolded by urea or temperature. The interaction of GuDCl with the peptide results in the separation of these resonances in the spectrum (Fig. 4). The resonances listed as Ala I, II, III correspond to Ala 4, 5, 6 respectively (see Fig. 4 legend).

In S-peptide (1–20), as in C-peptide lactone¹², Ala 4, 5, and 6 show significant helix shifts; the difference between the GuDCl titration curves at 0° and at 37 °C is shown for each resonance in Fig. 4*c*. In marked contrast, Ala 19 and 20 do not show measurable helix shifts (Fig. 4*c*), supporting further the sugges-

Fig. 3 GuDCl titrations of S-peptide (1–20). *a*, 0 °C, pH 3.8, 0.1 M NaCl, D₂O. *b*, 47 °C, pH 3.8, 0.1 M NaCl, D₂O. *c*, $\Delta\delta$ (0–47 °C); the difference in chemical shift between 0 °C and 47 °C.

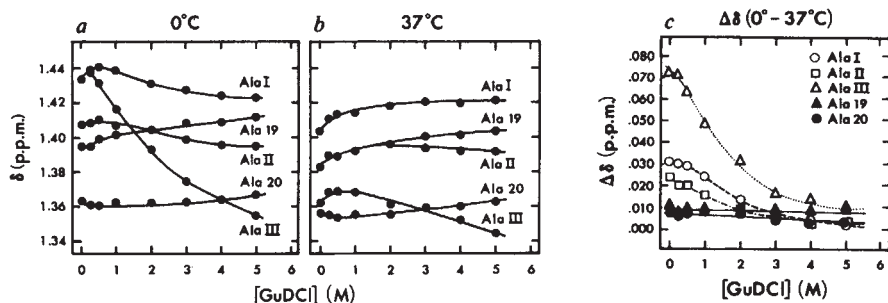
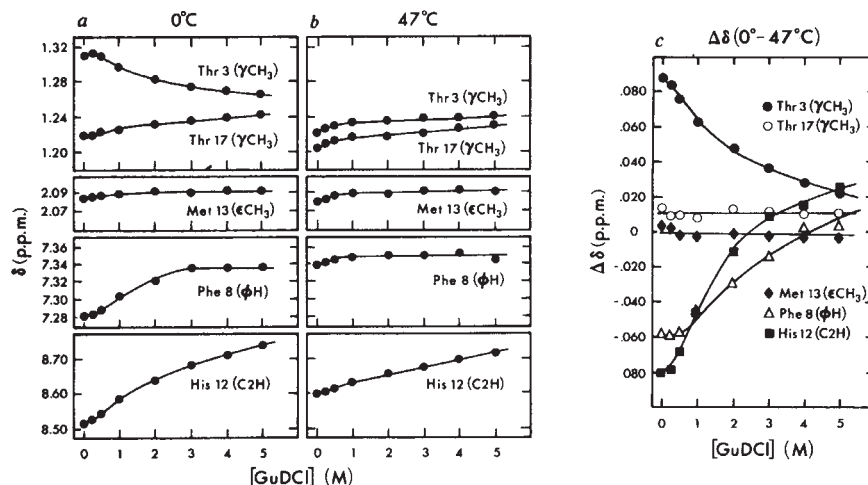
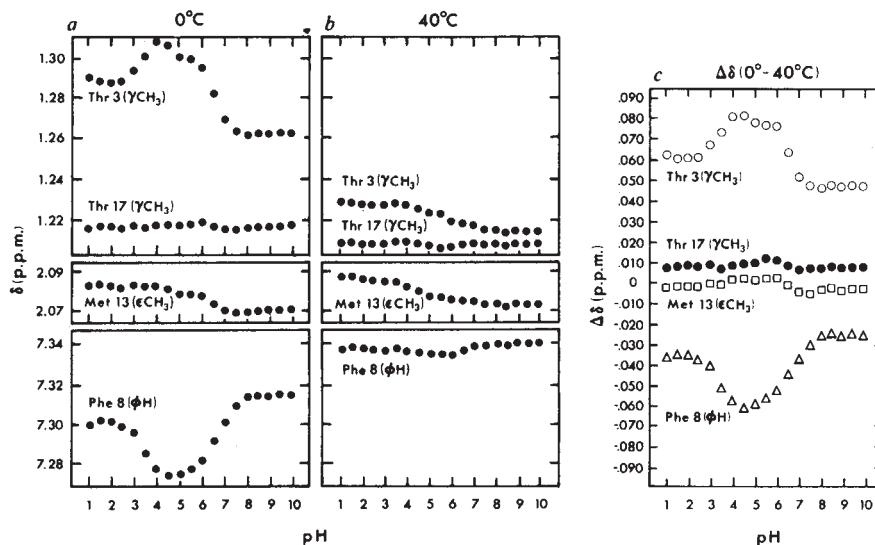


Fig. 4 GuDCl titrations of the alanine β -CH₃ resonances in S-peptide (1–20). *a*, 0 °C, pH 3.8, 0.1 M NaCl, D₂O. *b*, 37 °C, pH 3.8, 0.1 M NaCl, D₂O. *c*, $\Delta\delta$ (0–37 °C); the difference in chemical shift between 0 °C and 37 °C. The average chemical shift for each alanine β -CH₃ doublet is shown. The resonances numbered Ala I, II, III correspond to Ala 4, 5 and 6 respectively. These assignments were made, after submission of this paper, using a synthesized peptide containing deuterated alanine. The resonance assignments of Ala 19 and 20 were made by comparing the spectra of S-peptide (1–15) and S-peptide (1–20) in the unfolded state (5.0 M GuDCl, 0° or 37 °C) to locate Ala 19 and 20, and by pH titration of the α -COOH group of Ala 20, to locate Ala 20.

Fig. 5 pH titration of S-peptide (1–20). *a*, 0 °C, 0.1 M NaCl, D₂O. *b*, 40 °C, 0.1 M NaCl, D₂O. *c*, $\Delta\delta$ (0–40 °C); the difference in chemical shift between 0 °C and 40 °C. The pH titration of His 12 (C2H) is dominated by changes in chemical shift due to the titration of the imidazole ring and is not depicted. The Ala β -CH₃ resonances are not well separated in some conditions and are not shown.



tion that the α -helix formed by isolated S-peptide (1–20) is localized. The alanine β -CH₃ resonances are particularly useful indicators of helix formation. Only one bond separates the alanine β -CH₃ group from the peptide backbone. Moreover, rotation about this bond (C_{α} – C_{β}) is fast on the NMR time scale under all conditions investigated here. A small linear shift is observed for Ala 20 as GuDCl is added, but this shift is clearly distinct from the helix shifts observed for Ala 4, 5, and 6 (Fig. 4c). The linear GuDCl shift for Ala 20 is probably related to the fact that Ala 20 is the last residue in the peptide, and contains the α -COO[–] group.

Comparison with CD results

A direct comparison between the molar ellipticity change (222 nm) caused by helix formation for C-peptide lactone and for S-peptide (1–20) is not yet possible, for the following reasons. (1) Only partial helix formation has been observed for either peptide, even in the optimal helix-forming conditions. For C-peptide lactone, a helix content of 30% at pH 5.0, 0 °C, has been estimated from CD measurements. It is likely that, in these conditions, 30% of the molecules are helical and 70% are in a random coil conformation; almost all resonances of C-peptide lactone show shifts as the helix is formed¹¹ (A.

Bierzynski *et al.*, manuscript in preparation). Any comparison between the CD changes on helix formation for the two peptides depends critically on the relative stabilities of the helices formed by the two peptides. (2) The pH-stability profiles for C-peptide lactone¹¹ and for S-peptide¹³ differ in important respects: the optimal pH is 5.0 for C-peptide lactone but 3.8 for S-peptide. Consequently, a comparison between helix stabilities at any given pH is arbitrary. A comparison between the molar ellipticity changes on helix formation by the two peptides has been made earlier, at pH 5.5, by Brown and Klee¹⁵, who were not aware either of the strong pH dependence of helix stability or of the different pH-stability profiles of the two helices. Also, they studied helix formation in an unresolved mixture of C-peptide lactone and C-peptide carboxylate, without knowing the striking difference between the helix-forming properties of these two peptides (contrast ref. 13).

We have made an approximate comparison between the molar ellipticity changes on helix formation for C-peptide lactone and S-peptide (1–20) in the following way. We assume that the residues within the helix have the same structure in the helical conformation of both peptides and consequently that the helix shifts for 100% helix formation are the same for both helices, measured for any of several side-chain resonances. From the observed helix shifts, we calculate a ratio for the extent of helix formation for the two peptides. This ratio is then compared to the ratio of ellipticity changes (per mole peptide). The results (Table 1) show that these two ratios are the same, indicating that approximately the same number of residues participate in helix formation in both peptides. This is consistent with the conclusion that the helix in S-peptide (1–20) terminates before Thr 17 and possibly near Met 13.

pH dependence of helix stability

As mentioned above, the stabilities of both the S-peptide (1–20) helix and the C-peptide lactone helix are strongly dependent on pH. However, the pH dependence of $[\theta]_{222}$ is more complex in S-peptide (1–20)¹³ than in C-peptide lactone¹¹. At low temperatures, where the helix is populated, the pH dependence of $[\theta]_{222}$ shows a complex curve¹³, in contrast with the almost symmetric bell-shaped curve found with C-peptide lactone¹¹. When either peptide is thermally unfolded (40–45 °C), $[\theta]_{222}$ is essentially independent of pH^{11,13}. These results suggest that charged residue(s) which affect helix stability exist in S-peptide (1–20) that are not present in C-peptide lactone.

We have monitored the pH dependence of chemical shift for some resonances of S-peptide (1–20) at 0 °C and 40 °C, and the results are depicted in Fig. 5. At 40 °C, some resonances show pH-dependent chemical shifts (Fig. 5b) that are unrelated to helix formation. We again take the difference in chemical shift between 0 °C and 40 °C, to obtain the shifts that are due to helix formation. The results (Fig. 5c) show that both Thr 3 and Phe 8 give pH-dependent helix shifts that are qualitatively similar to the pH dependence of $[\theta]_{222}$ for S-peptide (1–20)¹³. In contrast, Thr 17 and Met 13 (S-CH₃) do not show a significant pH-dependent shift in the temperature difference plot (Fig. 5c). These data agree with the conclusion that S-peptide (1–20) contains a functional helix termination signal, and they suggest that the same residues are contained within the helix at all pH values where helix formation is observed.

NMR and helix termination

In the present study, we have monitored side-chain resonances and have measured 'helix shifts' (changes in chemical shift caused by alterations in the helix stability with temperature, urea, GuDCI, or pH) to determine whether a given residue participates in helix formation. The fact that self-consistent results are obtained with all four methods of changing the helix content lends support to this procedure. Nevertheless, the results with the S-CH₃ resonance of Met 13 point out a possible danger in using this approach: the group may be too far removed from the peptide backbone to show a significant change in chemical shift on helix formation. In this regard, the results with the

β -CH₃ resonances of Ala 4, 5, 6, 19, and 20 are particularly valuable in indicating helix termination within the peptide, because the β -CH₃ group of Ala is very close to the peptide backbone of the helix. In order to study these five Ala resonances, it was necessary to make use of the ability of GuDCI to spread out the chemical shifts of groups of the same residue type.

Helix termination signal

Measurements of the equilibrium constant for nucleation (σ) in homopolymers and random copolymers⁹ indicate that σ is always very small ($\sigma \sim 10^{-4}$). The cooperative length of an α -helix in aqueous solutions is thus predicted to be about 100 residues² ($=1/\sigma^{1/2}$). In contrast, our results indicate that the helix formed by S-peptide (1–20) is localized to certain residues. The features of the naturally occurring amino acid sequence of S-peptide (1–20) that are responsible for helix termination are of obvious interest.

In order to understand the mechanism of helix termination in S-peptide (1–20), we will need to have a more detailed description of termination. One wants to know at which residue the helix terminates and whether termination is gradual (spread out over a few residues) or abrupt. In future work it will be useful to use ¹³C-enriched amino acids to monitor backbone resonances, and to monitor additional side-chain resonances with two-dimensional NMR techniques. One would also like to find conditions (perhaps with new derivatives of the S-peptide and C-peptide) in which helix formation goes to completion for those residues in the helix. Then the molar CD changes for helix formation in different peptides can be compared directly, and the NMR results can be extended by measuring amide proton exchange rates and nuclear Overhauser effects.

We consider here two contrasting models for helix termination in peptides that may also play a role in protein folding. Our present data are largely consistent with a simple helix termination mechanism in which the amino acid sequence increases the helix nucleation constant (σ), and therefore decreases the cooperative length of the helix (for example, increasing σ to 10^{-2} would decrease the cooperative length to 10 residues). Then, a stretch of residues with low helix propensity (for example, residues 14–18 of S-peptide) would prevent propagation of the helix. The observed stabilities of the C-peptide lactone and S-peptide (1–20) helices are 1,000-fold greater than that predicted using the value of $\sigma \sim 10^{-4}$ obtained from polymer studies. If σ were larger in S-peptide, for example due to specific interactions between sidechains, then the increased stability and termination of the S-peptide (1–20) helix could be explained. On the other hand, the mechanism of helix termination may directly involve specific interactions between sidechain groups. For example, model building shows that a H-bonding interaction between the sidechains of His 12⁺ and Asp 14[−] would prevent propagation of the S-peptide (1–20) helix past residue 13. The pH dependence of S-peptide (1–20) is more complex than that of C-peptide lactone¹³, and the additional ionizing groups in S-peptide (1–20) involve only Asp 14 and the α -COO[−] group of Ala 20. More work is needed to distinguish between these and other possible explanations of helix termination in S-peptide.

Mechanism of protein folding

Studies of C-peptide lactone¹¹ and S-peptide¹³ have demonstrated that isolated units of secondary structure can have significant stability in water. The data presented here suggest that functional termination signals can exist in isolated units of secondary structure. These results are consistent with the simple framework model for protein folding in which the amino acid sequence directly determines the positions of the α -helices and β -sheets, and these secondary structures then pair to form the tertiary fold. Since C-peptide lactone and S-peptide provide the first examples of α -helix formation and termination in aqueous solutions of a short peptide fragment of a protein, it is important to point out that they provide only isolated examples, and many

more examples will be needed to base conclusions about the folding mechanism.

These data are encouraging with regard to predicting secondary structure from amino acid sequence because they suggest that helix formation in the isolated S-peptide is localized to the same, or nearly the same, residues as in native RNase A. It is interesting that present schemes for predicting secondary structure are more successful in finding the locations of helices than in defining their endpoints¹⁷. Experiments of the type described here, with peptides of specified amino acid sequence, may provide clues about the nature of helix termination signals.

We thank Drs K. Kuwajima and D. E. Wemmer for discussions, S. G. Boxer for use of his computing facilities and V. MacCosham for technical assistance. This work was supported by NIH grant 2 RO1 GM 19988-22. P.S.K. is a predoctoral fellow of the Medical Scientist Training Program. Use of the Stanford Magnetic Resonance Facility (supported by NSF grant GP 23633 and NIH grant RR 00711) is acknowledged.

Note added in proof: A recent ¹H-NMR study²⁵ (published after submission of this article) of helix formation by S-peptide (1–19) agrees with our conclusion that the helix is limited to certain residues including the helical residues of RNase A.

Received 5 September; accepted 10 October 1983.

- Schulz, G. E. & Schirmer, R. H. *Principles of Protein Structure* (Springer, New York, 1979).
- Poland, D. & Scheraga, H. A. *Theory of Helix-Coil Transitions in Biopolymers* (Academic, New York, 1970).
- Wlodawer, A., Bott, R. & Sjölin, L. *J. biol. Chem.* **257**, 1325–1332 (1982).
- Richards, F. M. & Wyckoff, H. W. *Atlas of Molecular Structures in Biology I. Ribonuclease-S* (Clarendon, Oxford, 1973).
- Richards, F. M. & Vithayathil, P. J. *J. biol. Chem.* **234**, 1459–1465 (1959).
- Wyckoff, H. W. *et al. J. biol. Chem.* **245**, 305–328 (1970).
- Epand, R. M. & Scheraga, H. A. *Biochemistry* **7**, 2864–2872 (1968).
- Taniuchi, H. & Anfinsen, C. B. *J. biol. Chem.* **244**, 3864–3875 (1969).
- Scheraga, H. A. *Pure appl. Chem.* **50**, 315–324 (1978).
- Brown, J. E. & Klee, W. A. *Biochemistry* **10**, 470–476 (1971).
- Bierzynski, A., Kim, P. S. & Baldwin, R. L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2470–2474 (1982).
- Bierzynski, A. & Baldwin, R. L. *J. molec. Biol.* **162**, 173–186 (1982).
- Kim, P. S., Bierzynski, A. & Baldwin, R. L. *J. molec. Biol.* **162**, 187–199 (1982).
- Schellman, J. A. *J. chem. Phys.* **62**, 1485–1494 (1958).
- Brown, J. E. & Klee, W. A. *Biochemistry* **8**, 2876–2879 (1969).
- Blackburn, P. & Moore, S. *The Enzymes* Vol. 15, 317–433 (Academic, New York, 1983).
- Schulz, G. E. *et al. Nature* **250**, 140–142 (1974).
- Garel, J.-R. *Eur. J. Biochem.* **70**, 179–189 (1976).
- Doscher, M. S. *Meth. Enzym.* **11**, 640–648 (1967).
- Kuwajima, K. & Baldwin, R. L. *J. molec. Biol.* **169**, 281–297 (1983).
- DeMarco, A. *J. Mag. Res.* **26**, 527–528 (1977).
- Ferrige, A. G. & Lindon, J. C. *J. Mag. Res.* **31**, 337–340 (1978).
- DeMarco, A. & Wüthrich, K. *J. Mag. Res.* **24**, 201–204 (1976).
- Chen, Y.-H., Yang, J. T. & Chau, K. H. *Biochemistry* **13**, 3350–3359 (1974).
- Rico, M. *et al. FEBS Lett.* **162**, 314–319 (1983).

Activation of a translocated human *c-myc* gene by an enhancer in the immunoglobulin heavy-chain locus

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A tissue-specific transcriptional enhancer element that is associated with the human immunoglobulin heavy-chain locus is defined. In a non-Hodgkin's lymphoma that contains a translocated c-myc gene this enhancer is retained on the 14q⁺ chromosome and occurs within sequences shown to activate previously cryptic promoters of the c-myc gene.

A FUNCTIONAL immunoglobulin gene is generated somatically during the differentiation of B lymphocytes by a set of developmentally regulated gene rearrangements¹. The major function of these rearrangements is to generate a diverse set of complete immunoglobulin genes from a limited number of inherited gene segments.

Another important role of these rearrangements has recently been identified at least for the mouse immunoglobulin heavy-chain genes^{2–4}. In an individual B lymphocyte and its progenies, only one of a few hundred copies of the variable (V) gene segments is expressed. This activation of a specific V gene segment results from its direct rearrangement into the vicinity of a transcriptional enhancer element that is located upstream of the constant (C) region gene segments, between the joining (J) segments and the switch (S) region. Interestingly, the immunoglobulin gene-associated enhancer functions in a tissue-specific manner, suggesting a more general involvement of cellular enhancer elements in tissue-specific expression of eukaryotic genes during cell differentiation.

Another biological process in which gene rearrangements and enhancer elements may play a critical, albeit adventitious, role is tumorigenesis. A model supporting this notion comes from the studies on the chicken leukaemogenesis induced by the non-acute avian leukosis virus (ALV). By integrating close to the cellular oncogene, *c-myc*, the ALV provirus via its transcriptional promoter or enhancer element can lead to increased levels

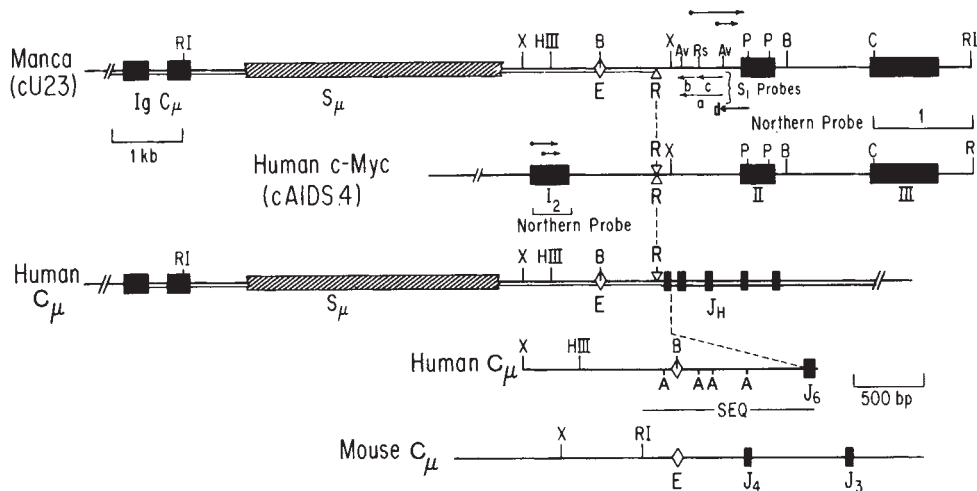
of *c-myc* transcription^{5,6}. It has recently been shown that the *c-myc* gene is translocated into an immunoglobulin locus in certain lymphoid neoplasms of both mice and men^{7–12}. Thus, murine *c-myc* gene is recombined into the immunoglobulin heavy-chain locus in BALB/c plasmacytomas characterized by t(12:15) translocations^{7,9–11}. Likewise, in a majority of the many Burkitt's lymphomas or non-Hodgkin's lymphomas characterized by t(8:14) (q24; q32) translocations, the *c-myc* gene is recombined into the immunoglobulin heavy-chain locus^{8,11,12}. It has generally been considered that in these and other neoplasms where nonrandom chromosomal translocations have been observed, *c-onc* genes are activated by the translocations^{13–15}.

To understand the relationship between the translocation and activation of a *c-myc* gene we have been analysing the structure and expression of normal and translocated human *c-myc* genes. The results presented here demonstrate that the human immunoglobulin heavy-chain locus carries a transcriptional enhancer element analogous to its mouse counterpart, and that this enhancer element may be playing a direct part in the activation of the translocated *c-myc* gene in some lymphoid neoplasms.

c-myc translocation in Manca cells

The non-Hodgkin's lymphoma, Manca¹⁶, shows a chromosome translocation (t(8:14)(q24;q32)) that is characteristic of many

Fig. 1 A comparison of the human *c-myc* gene and the C_{μ} region of the human immunoglobulin heavy-chain locus to the C_{μ} -*c-myc* joining region of the 14:8 translocated chromosome from Manca cells. Exons are shown as solid boxes; chromosome 14 sequences as a double line; and chromosome 8 sequences as a single line. R indicates the site of recombination between the two chromosomes. E is a transcriptional enhancer element, which was previously identified for the mouse immunoglobulin heavy-chain locus²⁻⁴ and the identification of the human equivalent is reported here. S_{μ} , C_{μ} switch sequences; J_{μ} , joining segments. The J_{μ} segments are defined on the basis of data presented in ref. 40, and our own sequencing⁴¹. The horizontal arrows indicate the initiation sites and directions of transcription. Restriction sites: RI, *Eco*RI; X, *Xba*I; H, *Hind*III; Av, *Ava*I; Rs, *Rsa*I; P, *Pst*I; B, *Bgl*II; C, *Cla*I; A, *Alu*I (the complete map is only shown for the first three of these). 1 and 2 delineate the extent of the probes used in the Northern blot experiments. The cloning of the recombination region of the translocated chromosome into cosmid pTCF¹⁹ will be described elsewhere (K.W. *et al.*, manuscript in preparation). The unrearranged *c-myc* gene was also cloned into a cosmid. Colonies were screened with the Northern probe 1. The definition of the *c-myc* gene will be described²².



Burkitt's lymphomas^{14,15}. *In situ* hybridization of a *v-myc* probe¹⁷ to Manca chromosomes indicates that the human *c-myc* locus is on that portion of chromosome 8^{12,18} that is translocated (data not shown).

From a cosmid library¹⁹ of Manca DNA a clone, cU23 was isolated that hybridized both to *v-myc* and to pH18-C1-10, a plasmid containing the immunoglobulin heavy-chain C_{μ} gene and sequences immediately 5' to it²⁰. Southern blot analysis²¹ of Manca DNA indicated that the linkage of *c-myc* and immunoglobulin heavy-chain gene sequences detected in cU23 had occurred in the human cells, and not upon cloning (data not shown). A detailed map of clone cU23 in the region of *c-myc*- C_{μ} linkage was derived by restriction analysis and DNA sequencing (K. W. *et al.*, manuscript in preparation), and was compared with maps of human germ-line C_{μ} and *c-myc* genes, contained on plasmid pH18-C1-10 and cosmid cAIDS4 respectively (Fig. 1). The latter clone was described previously²². It can be deduced that in Manca DNA, the human *c-myc* gene is fused head-to-head with the C_{μ} gene, about 6.5 kilobases (kb) 5' to the first C_{μ} exon. The junction on chromosome 14 lies between S_{μ} and $J_{H}6$, at a point that is not normally used for the productive rearrangement of an immunoglobulin heavy-chain gene. The junction on chromosome 8 occurs between exons I and II of the *c-myc* gene. As a result, the Manca DNA cloned in cU23 contains neither exon I nor the normal transcriptional start sites²² of the human *c-myc* gene. Instead, *c-myc* exons II and III are fused to C_{μ} sequences that include a stretch of DNA between S_{μ} and J_{H} that is in an analogous location to sequences in the mouse genome shown to have tissue-specific transcriptional enhancing activity²⁻⁴ (region 'E' in Fig. 1). The translation of transcripts of the human *c-myc* gene begins within exon II, at an initiation AUG codon that is retained, and presumably used, in transcripts of the translocated *c-myc* gene in Manca cells.

Multiple transcription initiation sites

Northern blot analysis of RNA from Manca cells indicates a high level of *c-myc* transcription (Fig. 2A). It is clear that there are multiple transcripts hybridizing to the *c-myc* Northern probe 1 (see Fig. 1). The level of *c-myc* transcription in Manca cells is the highest that we have detected in any cell line (which include other Burkitt's lymphomas, and Epstein-Barr virus-immortalized lymphoblastoid cells) except the promyelocytic leukaemia cell line HL60 (data not shown). The relevance of this comparison is unclear, however, as all these cells are growth

transformed. An alternative approach is to compare the level of transcription of the rearranged *c-myc* gene in Manca cells with that of the unrearranged allele; with a probe specific for exon I no transcription of the unrearranged *c-myc* gene can be detected in Manca cells (K.W. *et al.*, manuscript in preparation). Therefore, the high *c-myc* level of transcription in Manca cells all derives from the translocated allele.

The initiation points of *c-myc* transcripts in Manca cells were mapped by S_1 nuclease protection experiments^{23,24} using the probes shown at the bottom of Fig. 2. The major initiation site (site 1; see Fig. 2) for *c-myc* transcripts occurs about 636 base pairs (bp) upstream of *c-myc* exon II, within what would ordinarily be intron I of the *c-myc* gene. The nucleotide numbering system used to denote the initiation points is that of Colby *et al.*²⁵, whose sequence begins within intron I of the human *c-myc* gene such that nucleotide 1,000 defines the start of exon II. Transcripts initiating at site I (nucleotide 364) form S_1 -resistant hybrids with probes b and a of 66 base pairs (bp) and 359 bp respectively (Fig. 2B, C). Downstream of site I, at nucleotide 378, is a weaker initiation point, Ia (Fig. 2B, C). As has been previously noted²⁵, upstream of initiation sites I and Ia are sequences related to the consensus TATA box^{26,27} commonly found 5' of genes transcribed by RNA polymerase II (see Fig. 3). Initiating at around nucleotide 873 are further transcripts, which form S_1 nuclease-resistant hybrids of length 192 bp with probe d (data not shown), and 28 bp upstream of this site occurs the sequence TTTATT (Fig. 3). In addition to these initiation sites, an S_1 sensitive site (X) in hybrids between Manca RNA and either probe a or c is detected at around nucleotide 520. However, the assignment of an initiation site to this position is uncertain, an S_1 -sensitive site also being apparent at this position in hybrids between probes a or c and yeast RNA on long exposures.

If transcripts initiating at these sites were to have no functional splice sites 5' of the junction between exon II and the following intron, then the processed RNAs would range in size from 1,660 to 2,450 nucleotides (not including the poly(A) tail²⁸). That this is the case is suggested by cDNA cloning, which has revealed a contiguous transcript extending upstream from exon II at least as far as position 660. Beyond this point the S_1 nuclease-sensitive sites have been confirmed as initiation (as opposed to splice) sites by primer extension analyses. For example, reverse transcripts of Manca cell RNA using the primer shown in Fig. 2B extend as far as nucleotide 364. Further primer extensions (for example using S_1 probe b) indicate no initiation sites upstream of those characterized here (data not shown).

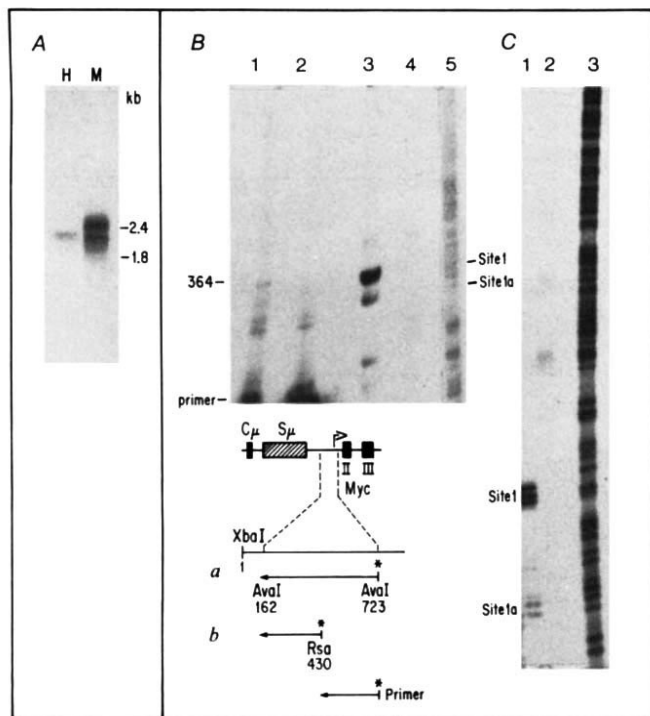


Fig. 2 **A**, Northern blot analysis⁴² using the probe 1 shown in Fig. 1 of mRNA from: lane H, HeLa cells (1 μ g); lane M, Manca cells (0.3 μ g). **B**, Lanes 1 and 2 show a primer extension analysis⁴³, and lanes 3 and 4 an S_1 nuclease mapping analysis^{23,24} of: lanes 1 and 3, Manca cell mRNA; lanes 2 and 4, yeast mRNA. The probes used are 'a' and 'b' shown in the diagram below the photograph of the blot, and were strand separated and end labelled⁴¹ before hybridization. The nucleotide numbering system derives from ref. 25, and starts (nucleotide 1) at the *Xba*I site in intron 1 of the human *c-myc* gene. Sites 1 and 1a are referred to in the text; site 1 centres on nucleotide 364, and the mark indicating this position on the right of the figure shows the correspondence between the primer extension and S_1 signals in lanes 1 and 3. Lane 5 is a pyrimidine analysis⁴¹ of the sense strand of the probes, from which the nucleotide positions of the DNA-RNA discontinuity could be determined (see Fig. 3). **C**, S_1 nuclease analysis using the probe 'b' shown in the diagram below **B** of mRNA from: lane 1, Manca cells; lane 2, yeast cells. Lane 3 is the same type of reaction as shown in lane 5 of **B**, but using probe b.

Methods: The S_1 nuclease reactions contained either 100 ng Manca cell mRNA plus 20 μ g yeast RNA, or only the yeast RNA. For the S_1 analysis 5×10^4 c.p.m. of probe was used per reaction, and for the primer extension analysis 2×10^4 c.p.m. of probe was used per reaction. The probes were annealed to the RNA at 53 °C for 4 h (except in the case of probe b, which was annealed at 30 °C for 12–16 h). S_1 reactions were performed at 15 °C for 3 h, and primer extension reactions at 41 °C for 45 min. A 6% acrylamide gel was used for **B**, and run for 7 h, and an 8% gel run for 3 h for **C**.

Transcriptional enhancer element

We have previously shown that sequences derived from the J_H - C_μ region of the mouse immunoglobulin heavy-chain locus contain a DNA element that enhances the transcription of the associated gene in a manner largely independent of both position and orientation². The translocation of the *c-myc* gene in Manca cell DNA just 300 bp downstream of the human J_H cluster (Fig. 1) suggested that the high level of *c-myc* transcription in Manca cells (Fig. 2A) might be due to a human enhancer element located in an analogous region to that in the mouse ('E' in Fig. 1).

To test this hypothesis, we first attempted to identify the putative human enhancer element. For this purpose, the 2.2 kilobase (kb) *Xba*I fragment (see Fig. 1) containing most of the sequence between the J_H cluster and S_μ , plus about 330 bp derived from the 5' flanking region of the translocated, truncated *c-myc* gene, was inserted into the *Eco*RI site of plasmid pSER². This plasmid was derived from pSV2.gpt²⁹ (which contains the mycophenolic acid-resistance conferring gene *Ecogpt*) by deletion from the latter of the SV40 enhancer sequences. As a result, pSER transforms cells to mycophenolic acid resistance (gpt⁺ phenotype) at a much lower frequency than does pSV2.gpt (see Table 1). The original level of transformation can be restored by the insertion into the *Eco*RI site of pSER of the mouse immunoglobulin heavy-chain locus enhancer element². As shown in Table 1, the insertion in either orientation of the human *Xba*I fragment into the *Eco*RI site of pSER (2.3 kb upstream of the *Ecogpt* gene) restored the gpt⁺ transformation efficiency to a level even exceeding (by about eightfold) that of pSV2.gpt. The transformation efficiency of pSV2.gpt was itself increased 5–10-fold by insertion of the *Xba*I into its *Eco*RI site. Other fragments of mouse and human immunoglobulin heavy-chain loci DNA had no significant effects on transformation frequencies when inserted into pSER of pSV2.gpt. Also, like its murine counterpart the effect of the *Xba*I fragment was tissue-specific: with rodent fibroblasts the transformation efficiencies of pSERXa and pSERXb were over 100 times lower than that of pSV2.gpt. In a further experiment the 2.2 kb *Xba*I fragment was dissected by cleavage with *Alu*I, and some of the resulting fragments also tested in the pSER assay. Most, if not all the enhancing activity could be attributed to the 279 bp fragment, *Alu*a (delineated by the two *Alu*I sites flanking the *Bgl*II site shown in the expanded map of the human C_μ gene in Fig. 1).

These transformation experiments strongly suggest that the 2.2 kb human *Xba*I fragment contains a tissue-specific transcriptional enhancer element. To demonstrate this point more directly we examined the effect of this DNA fragment on the transcription of a mouse immunoglobulin γ 2b gene. The plasmid pSV. γ 2b Δ X_{2/4} contains a functionally rearranged mouse γ 2b gene from which most of the sequences (including the enhancer element) between *V-D-J* and *C* exons have been deleted². The plasmid also contains the selectable *Ecogpt* gene. J558L cells transformed to mycophenolic acid resistance by pSV. γ 2b Δ X_{2/4} express almost no γ 2b heavy-chain RNA². A high level of γ 2b expression can be restored by re-insertion (either upstream or downstream of the *V-D-J* promoter) into plasmid pSV. γ 2b Δ X_{2/4} of the mouse enhancer², and similarly by insertion of the human *Xba*I fragment (Fig. 4). In this experiment, pools of cells transformed to a gpt⁺ phenotype by pSV. γ 2b Δ X_{2/4} and its derivatives were assayed for γ 2b gene expression by S_1 nuclease protection. The mouse enhancer was equally effective whether inserted upstream or downstream of the γ 2b *V-D-J* promoter. By contrast the level of the γ 2b gene transcription attained by the human enhancer was somewhat variable depending on its position, the highest level of expression being obtained when the enhancer was inserted upstream of the promoter in the opposite orientation to the direction of transcription (Fig. 4). Interestingly, the relative orientations of enhancer and promoter in this case are equivalent to those of the enhancer and the altered *c-myc* promoters in Manca cell DNA (see Fig. 1).

The nucleotide sequence of a portion of the J_H - C_μ region of the human germ-line clone pH18-CL-10 was determined and compared with that of the corresponding region of mouse DNA (Fig. 5), which contains the enhancer core sequences³⁰ which are common to enhancers so far defined. Core-like sequences are present in the human DNA: there is a 67% matching between the 140 bp of mouse DNA that contains most, if not all, of the murine enhancing activity and the corresponding human sequence. The similarity between the mouse and human sequences throughout the region shown in Fig. 5 is sufficiently strong to allow a unique alignment, with several insertions and deletions.

Activation of *c-myc* transcription

To begin to investigate whether the high level of *c-myc* transcription in Manca cells was due to proximity to the immunoglobulin

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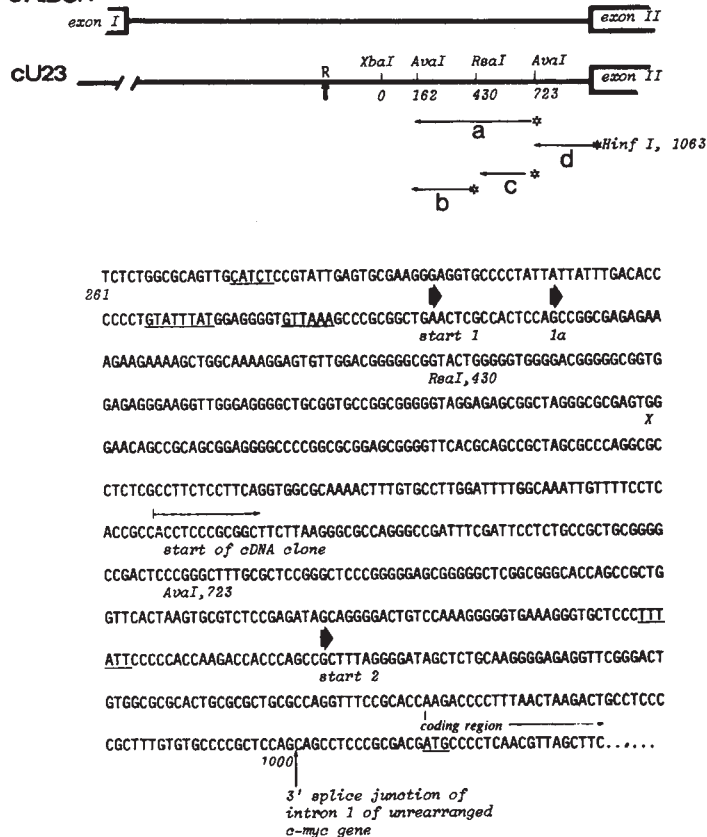


Fig. 3 Shows the nucleotide sequence from intron I of the germ-line *c-myc* gene that contains the initiation sites of the transcripts of the translocated *c-myc* gene. The recombination site of the translocated Manca chromosome is 336 nucleotides 5' of the start of the sequence shown. Exon II of the germ-line *c-myc* gene starts at nucleotide 1,000. The transcription initiation sites (1, 1a and 2), as deduced by S_1 and primer extension analyses, are marked by the thick arrows. The probes used in these analyses (a–d, a and b being those used in Fig. 2) are shown near the top of the figure. The asterisks mark the 32 P-labelled ends of the probes. Underlined sequences are similar to those commonly found 5' of RNA polymerase II-transcribed genes²⁷. The initiator ATG codon of the protein encoded by *c-myc* is also underlined. X is an S_1 -sensitive site, and R the recombination site. The sequence data derive from ref. 25 plus our own data in the regions of designated promoters.

heavy-chain locus enhancer, the transcription of the translocated *c-myc* gene was studied in a murine myeloma cell line, J558L. The myeloma cells were transformed by spheroplast fusion with either of two plasmids, pSV.26 or pSV2.Δ4. Plasmid pSV2.26 contains the 11.7 kb *EcoRI* fragment from cU23 (containing the translocated *c-myc* gene plus about 8 kb of 5' flanking sequences which include the immunoglobulin heavy-chain locus enhancer, see Fig. 1) the translocated *c-myc* gene cloned into the *EcoRI* site of pSV2gpt. Plasmid pSV2Δ4 contains a 9.5 kb *EcoRI* fragment (derived from the 11.7 kb *EcoRI* fragment of cU23 by deletion of the central 2.2 kb *XbaI* fragment) cloned into the *EcoRI* site of pSV2gpt. Plasmid pSV2Δ4 thus lacks the sequences identified above as having transcriptional enhancing activity.

After transforming with these plasmids, gpt⁺ transformants of J558L cells were isolated and assessed for the presence of exogenous DNA. Clones 26.1–26.9 were nine cloned isolates of cells transformed by pSV2.26; Δ4.2 is a cell line developed after transformation with pSV2.Δ4; Δ4.1 is a pool of several colonies transformed by pSV2.Δ4. Most cells transformed by pSV2.26 have acquired only single or a few copies of the exogenous DNA, and in most of these cases the integration has

Table 1 Transformation efficiency of pSV2.gpt and derivative plasmids

Plasmid	Frequency of transformation to gpt ⁺ phenotype	
	J558L cells*	rat-2 cells†
pSV2.gpt	1×10^{-4}	5×10^{-3}
pSV2.gpt.Xa‡	6×10^{-4}	ND
pSER	5×10^{-6}	ND
pSER.Xa‡	8×10^{-4}	1×10^{-5}
pSER.Xb‡	8×10^{-4}	1×10^{-5}
pSER.Ra§	8×10^{-6}	ND
pSER.Alu.a	2×10^{-4}	ND
pSER.Alu.z	5×10^{-6}	ND

Escherichia coli cells containing the above-mentioned plasmids were converted to spheroplasts³⁹, and fused² to recipient cells (10^{10} spheroplasts per 2×10^6 cells), after which the cells were plated at 5×10^3 (J558L) cells per well or 1×10^5 (rat-2) cells per culture dish. The selective medium, which contained xanthine ($250 \mu\text{g ml}^{-1}$), hypoxanthine ($15 \mu\text{g ml}^{-1}$) and mycophenolic acid ($6.5 \mu\text{g ml}^{-1}$), was added 48 h after fusion, and colonies were counted after 14 days. The experiments were routinely repeated three times. ND, not done.

* A mouse plasmacytoma cell line.

† A rodent fibroblast cell line.

‡ Xa/Xb plasmids contain an insertion in either orientation (a or b) of the 2.2 kb *XbaI* fragment from cU23.

§ pSER.Ra contains an insertion of a 4 kb *EcoRI* fragment from the mouse immunoglobulin heavy-chain C_{α} gene.

|| Alu.a/Alu.z plasmids contain an insertion of either of two *AluI* fragments (a and z) derived from the 2.2 kb *XbaI* fragment.

occurred within the 11.7 kb *EcoRI* fragment, so that *EcoRI* fragments smaller than 11.7 kb are detected with the *c-myc* probe³¹. Analysis with enzymes that cut within this fragment, however, indicate that in at least four out of nine cases (clones 26.1, 26.2, 26.4 and 26.7) contiguous DNA sequences that extend from the enhancer through the transcriptional initiation sites mapped above, and into the truncated *c-myc* gene, are intact (Fig. 6).

By contrast, the cells transformed in identical conditions by pSV2.Δ4, contain multiple, tandem copies of the exogenous DNA, and in each case the *c-myc* transcription unit and upstream sequences are intact. This difference in copy number of transforming DNA in cells selected for gpt⁺ phenotype may be due to a positive effect (from over 7 kb away) of the enhancer on *Ecogpt* transcription: in the absence of the enhancer, high levels of *Ecogpt* transcription may only be obtained from an increased copy number. Consistent with this, the transformation frequency of J558L cells by pSV2Δ4 is 5–10-fold lower than the frequency of transformation by pSV2.26 (data not shown). The difference in copy number may also result from a deleterious effect of excess *c-myc* gene product on J558 cells, which already contain an actively transcribed translocated mouse *c-myc* gene³². If such an effect exists, and if the immunoglobulin heavy-chain locus enhancer does activate Manca cell *c-myc* transcription, then there may be a selective advantage to cells transformed with pSV2.26 that retain only one or even no intact copies of the Manca cell *c-myc* gene.

Both total and poly(A)⁺ RNA were isolated from groups of transformants, and were analysed by Northern transfer and hybridization to probe 1 (see Fig. 1). Although transformants receiving pSV2.Δ4 contained multiple intact copies of the transcribed *c-myc* gene, none of them showed evidence for much *c-myc* transcription over and above the level of the mouse *c-myc* transcription endogenous to J558L cells (data not shown). By contrast several lines of cells transformed by pSV2.26 showed strongly hybridizing *c-myc* transcripts (data not shown).

Because of the variability in retention of *c-myc* DNA sequences, and the cross-reactivity of the Northern probe with the mouse *c-myc* transcripts in J558L cells, a quantitative com-

parison of human translocated *c-myc* gene transcription was better made by S_1 nuclease mapping. The S_1 probe *a* (Fig. 3), which detects the major initiation site, was protected to a similar quantitative degree by Manca cell RNA and by RNA of several lines transformed by pSV2.26 (for example, lines 26.1, 26.4 and 26.7, see Fig. 7). That RNA in these cell lines is qualitatively the same as in Manca cells was further indicated by use of S_1 probe *b*. Use of either probe shows almost no protection by RNA of cell lines $\Delta 4.1$ or $\Delta 4.2$ (see Fig. 7) even though these cells contain multiple intact copies of the transforming DNA

(Fig. 6). It cannot be argued that in either of these cases the transforming DNA has become sequestered in a transcriptionally inactive area, since continual selection for *Ecogpt* gene activity is made. In addition, several isolates of both total and poly(A)⁺ RNA from these cells gave the same result, and these RNAs were positive in an S_1 nuclease protection assay of *Ecogpt* transcripts (data not shown). It appears then, that the activity of the major transcriptional initiation site for translocated *c-myc* gene transcription is severely reduced by deletion of sequences 360 bp upstream of it. Deletions at this distance upstream of a

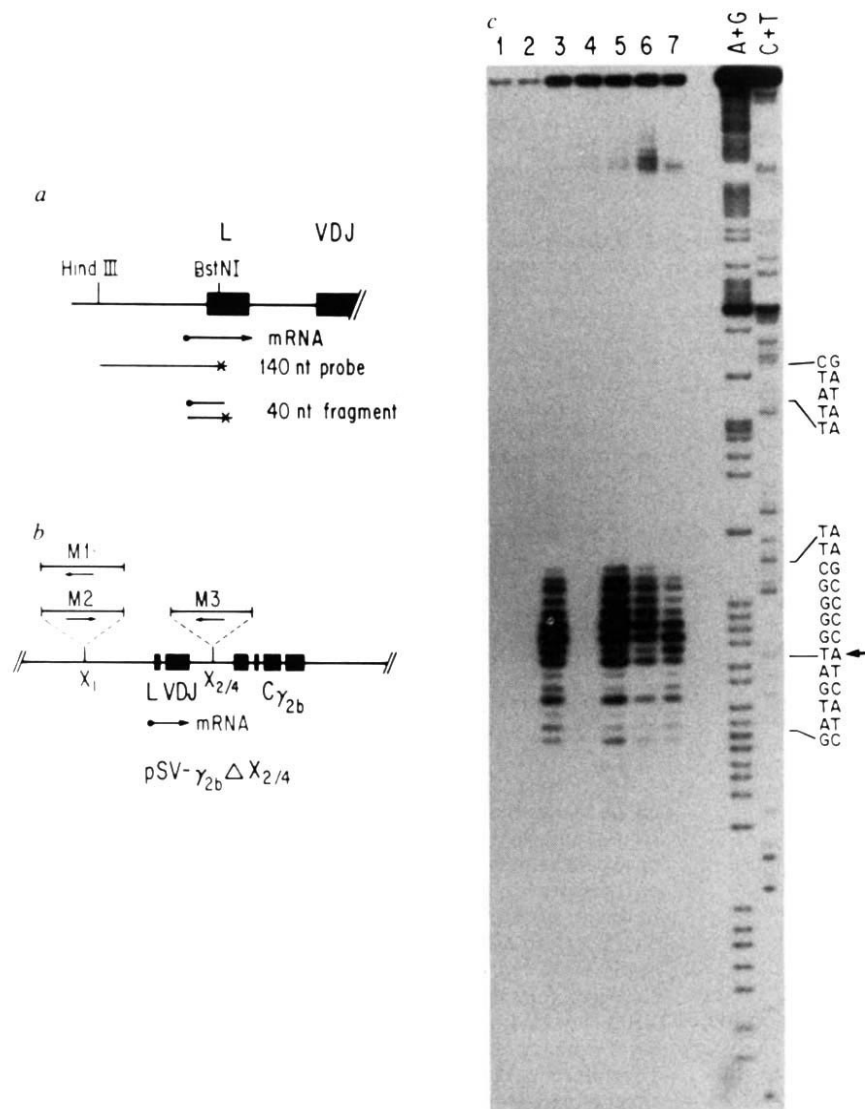


Fig. 4 *a*, Strategy of the S_1 nuclease mapping experiment shown in *c*. The probe is an end-labelled *HindIII*-*BstNI* fragment, which was strand separated and annealed to total cell mRNA before S_1 digestion and electrophoretic resolution, which were carried out as described previously^{23,24}. The 40 bp fragment protected by correctly initiated γ_{26} mRNA is as predicted by primer extension analysis⁴⁴. The solid blocks indicate the leader (L) exon and 5' end of the V-D-J exons. *b* Shows the derivatives of pSV- $\gamma_{2b}\Delta X_{2/4}$ used for transfection. The 2.2 kb *XbaI* fragment into the X₁ (M1 and M2) or X_{2/4} (M3) sites² in the indicated orientations. *c* Shows the S_1 nuclease resistant hybrids formed between the probe shown in *a* and: lane 1, 20 μ g tRNA; lane 2, 20 μ g RNA from J558L cells; lanes 3-7, 20 μ g RNA from J558L cells transfected with: lane 3, pSV- $\gamma_{2b}\Delta VC^2$; lane 4, pSV- $\gamma_{2b}\Delta X_{2/4}$; lane 5, pSV- $\gamma_{2b}\Delta X_{2/4}$ M1; lane 6, pSV- $\gamma_{2b}\Delta X_{2/4}$ M2; lane 7, pSV- $\gamma_{2b}\Delta X_{2/4}$ M3. In the two right-hand lanes are co-electrophoresed sequencing reactions³⁴ of the probe, which allow the identification of the transcription initiation site (arrow) and the GATAA box at position -30.

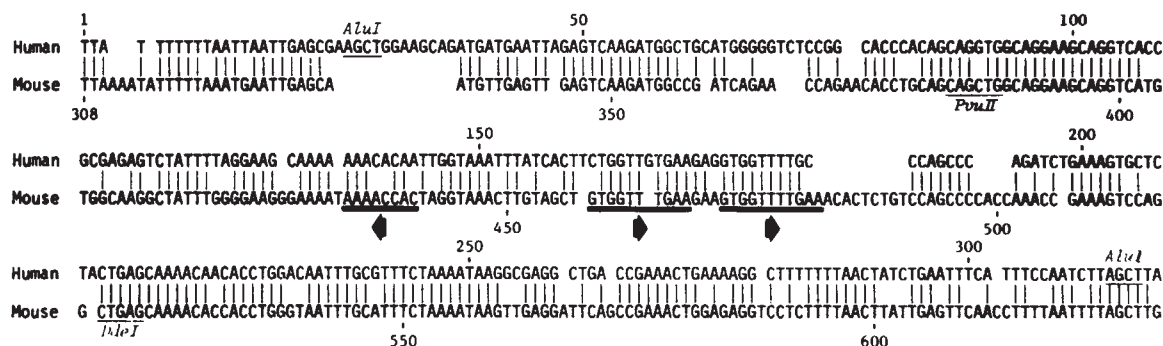


Fig. 5 Sequences of the murine² and human enhancer elements, which were compared using the SEQ computer program⁴⁵.

Fig. 6 Southern blot analyses of *c-myc* containing sequences in J558L cells, and in their derivatives transformed with either pSV2.26 or pSV2.Δ4. **a** Shows a blot obtained using probe 1 (as indicated in the diagram below) and *Hind*III plus *Eco*RI digested DNA from: lane 1, Δ4.2 cells; lane 2, Δ4.1 cells; lane 3, J558L cells; lane 4, 26.7 cells; lane 5, 26.2 cells; lane 6, 26.1 cells; lane 7, 5 pg of pSV2.26 cleaved with *Hind*III plus *Eco*RI, co-electrophoresed as a marker. **b** Shows a blot obtained using probe 3 and *Bgl*II plus *Eco*RI cleaved DNA from: lane 1, 26.1 cells; lane 2, 26.5 cells; lane 3, 26.4 cells; lane 4, J558L cells; lane 5, as in lane 7 of **a**, but cut with *Bgl*II. The diagram below the blots shows the 11.7 kb *Eco*RI fragment contained in pSV2.26 and also the position of the 6.5 kb *Hind*III-*Eco*RI fragment. The deletion (Δ) in pSV2Δ4 removes the *Hind*III site, as a result of which a 9.6 kb *Eco*RI fragment is the *Hind*III plus *Eco*RI digestion product that hybridizes to the *c-myc* probe. As a further result of this deletion the 3.1 kb *Bgl*II fragment detected with probe 3 (indicated in **b**) is only detected in pSV2.26 transformants. Bands labelled J in **a** are due to the cross-reactivity of probe 1 with normal and rearranged mouse *c-myc* genes in J558L cells^{11,32}. Restriction sites marked in the lower diagram: R, *Eco*RI; X, *Xba*I; H, *Hind*III; B, *Bgl*II; A, *Ava*I; C, *Cla*I.

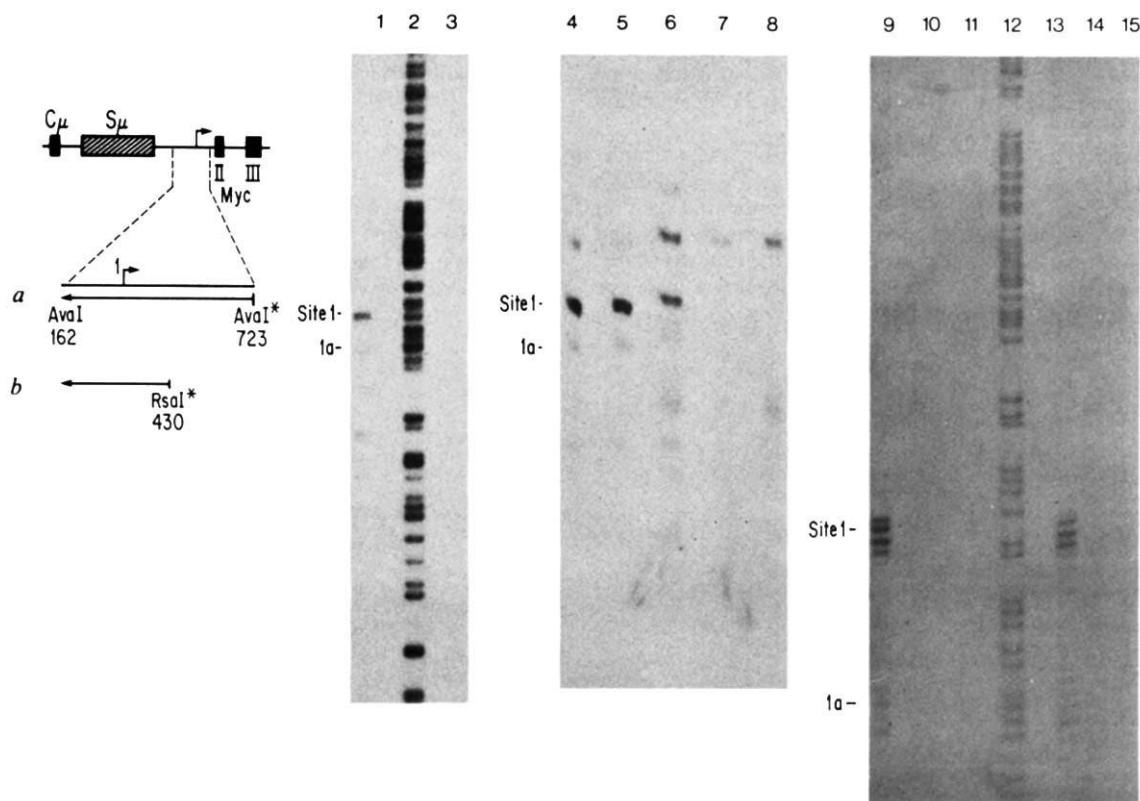
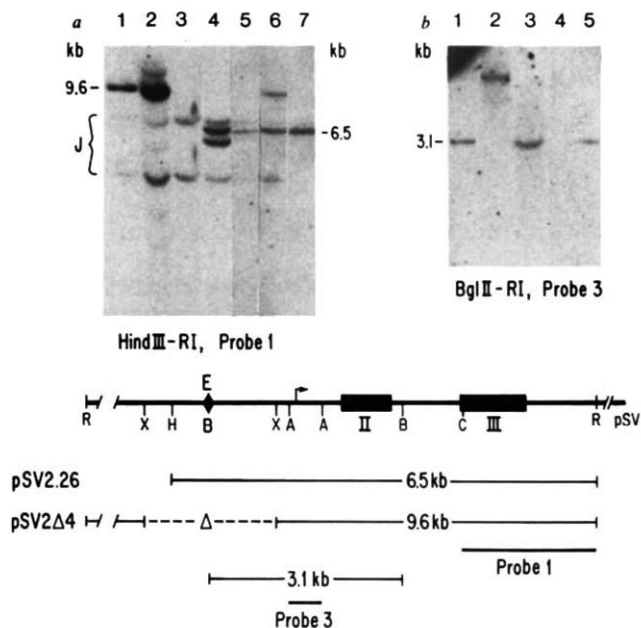


Fig. 7 S₁ nuclease analysis of mRNA from Manca cells, J558L cells, and transformed derivatives of J558L cells. Lanes 1–8 were reactions involving probe a, and lanes 9–15 involved probe b. Lane 2 is a pyrimidine sequencing reaction of probe a, and lane 12 is a similar reaction with probe b. The other lanes are the S₁ reactions, with mRNA from: lane 1, 26.7 cells; lane 3, J558L cells; lane 4, 26.1 cells; lane 5, 26.4 cells; lane 6, Manca cells; lane 7, Δ4.1 cells; lane 8, yeast cells; lane 9, Manca cells; lane 10, 26.3 cells (which retained no human *c-myc* sequences); lane 11, Δ4.1 cells; lane 13, 26.1 cells; lane 14, Δ4.2 cells; lane 15, J558L cells. The end-labelled, strand-separated⁴¹ probes were melted at 85 °C, then annealed to the RNAs at 51 °C for probe a, and at 30 °C for 12 h for probe b. S₁ digestions were carried out at 15 °C for 2 h. Electrophoresis was carried out as described previously, for lanes 1–8 on a 6% gel run for 7 h, for lanes 9–15 on an 8% gel run for 3 h. All reactions contained equal amounts of sample RNA made up to 20 μg with yeast RNA. S₁ nuclease-sensitive sites specific for the presence of lymphoid RNA are indicated.

promoter are characteristic of enhancer mutations³³. As the enhancing activity of the deleted fragment has been demonstrated and largely localized to one fragment (the *Alu* 'a' fragment), it appears likely that it is the removal of the immunoglobulin heavy-chain locus enhancer that has drastically reduced transcription of the translocated *c-myc* gene.

Multiple mechanisms of *c-myc* activation

We show here that Manca cells contain a translocated *c-myc* gene that is transcribed from previously cryptic start sites that ordinarily form part of intron I of the *c-myc* gene. This transcription is reproduced with fidelity after introduction of the gene into mouse myeloma cells. Use of this system has allowed us to demonstrate that transcription from at least the major cryptic promoter is dramatically dependent on the presence of sequences over 350 bp upstream of the RNA initiation site. These sequences include an element that we demonstrate to be a human immunoglobulin gene-associated enhancer element, and it may be this element itself that is responsible for activating translocated *c-myc* transcription in Manca cells. The remaining possibility, that it was the removal of other sequences (including about 300 bp of DNA that are also ordinarily part of *c-myc* intron I) that drastically reduced transcription from initiation site I is presently being investigated. The unrearranged *c-myc* gene is still present in Manca cells, but our preliminary results from use of Northern probes (probe 2 in Fig. 1), S₁ mapping and cDNA cloning indicate that this allele is barely, if at all, expressed. Such a situation has already been reported for a mouse plasmacytoma and may be true for other Burkitt's lymphomas^{11,32,34}. Hence, transcriptional activation of the *c-myc* gene appears to be an important result of translocation and in the case described here, it is possible that such activation is largely contributed to by the immunoglobulin heavy-chain locus enhancer.

The retention of the immunoglobulin associated enhancer element adjacent to the translocated *c-myc* gene is not a common feature of *c-myc* translocations so far documented in either mouse^{7,10,11,35} or man^{12,36}. In these instances, the translocated gene is still transcribed^{7,10,11,37}, whilst again the untranslocated gene may be silent^{11,32,34}. In these cases, transcription of the translocated *c-myc* gene may be maintained by a cellular enhan-

cer-type element yet to be identified, and which may be a considerable distance from the translocated *c-myc* gene.

Alternatively, translocated *c-myc* genes may not be directly dependent upon immunoglobulin sequences for their transcription, but rather their transcription may be activated by the accumulation of mutations in regulatory regions. For example, it has been suggested³⁴ that the *c-myc* gene product itself may regulate its own transcription, and that, in lymphomas harbouring translocated genes, the translocated but not the normal *c-myc* gene, escapes this repression. At this stage, the hypothesis of positive activation, and that of escaping repression, are not mutually exclusive.

The detachment of *c-myc* exon I sequences from *c-myc* exons II and III is a consequence for all BALB/c and for several human *c-myc* translocations thus far documented^{7,10-12,38}. We have previously suggested^{22,31} that this event, in itself, leads to increased translation of *c-myc* RNA in cells harbouring such translocations.

In summary, there are probably multiple steps in the activation of the human *c-myc* gene in non-Hodgkin's lymphomas. From this article it is clear that previously cryptic promoters of the *c-myc* gene are activated upon translocation, and that this activation is dependent upon upstream sequences. In Manca cells, the critical element in these upstream sequences may be the human immunoglobulin heavy-chain locus enhancer element. As both the translocated *c-myc* gene from a mouse plasmacytoma and the translocated human *c-myc* gene from Manca cells have detectable biological activity (ref. 46 and our unpublished data) it is apparent that the activation of the *c-myc* gene contributes to either initiation or maintenance of oncogenesis. It is possible that the misuse of a developmentally regulated, cellular enhancer element will be found, in many cases, to be responsible for activating oncogenes.

We thank Lena Angman, John McMaster, Anne Maxwell and Eleanor Basel for their help, and P. Jat for advice concerning S₁ analysis, C. M. Croce for the gift of pH18-cl-10, W. Topp for the kind distribution of rat-2 cells, and B. Clarkson for Manca cells. A.C.H. and K.W. thank ICRF and EMBO respectively for travel and long term fellowships. The work was supported by NIH grants AI-17879 (to S.T.), CA-14051 (a core grant to S. Luria), and CA-34502 (W.H.).

Note added in proof: The integrity of all RNA preparations has been confirmed by successful S₁ analysis of gpt transcription.

Received 17 October; accepted 30 November 1983.

1. Tonegawa, S. *Nature* **302**, 575-581 (1983).
2. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33**, 718-728 (1983).
3. Banerji, J., Olson, L. & Schaffner, W. *Cell* **33**, 729-740 (1983).
4. Neuberger, M. *EMBO J.* **2**, 1373-1379 (1983).
5. Hayward, W. S., Neel, B. & Astrin, S. *Nature* **290**, 475-480 (1981).
6. Payne, G. S., Bishop, J. M. & Varmus, H. E. *Nature* **295**, 209-215 (1982).
7. Shen-Ong, G. L., Keath, E., Piccoli, S. P. & Cole, M. D. *Cell* **31**, 443-452 (1982).
8. Dalla-Favera, R., Marinotti, S., Gallo, R., Erikson, J. & Croce, C. M. *Science* **219**, 963-967 (1983).
9. Crews, S., Barth, R., Hood, L., Prehn, J. & Calame, K. *Science* **218**, 1319-1321 (1982).
10. Marcu, K. H. *et al. Proc. Natn. Acad. Sci. U.S.A.* **80**, 519-523 (1983).
11. Adams, J. M., Gerondakis, S., Webb, E., Corcoran, L. M. & Cory, S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1982-1986 (1983).
12. Taub, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7837-7841 (1982).
13. Cairns, J. *Nature* **289**, 353-357 (1981).
14. Klein, G. *Nature* **294**, 313-318 (1981).
15. Rowley, J. D. *Science* **216**, 749-751 (1982).
16. Nishikori, M. *et al. Cancer Genet. Cytogenet.* (in the press).
17. Lautenberger, J. A., Schulz, R. A., Garon, C. F., Tschlis, P. N. & Papas, T. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1518-1522 (1981).
18. Neel, B., Jhanwar, S., Chaganti, R. & Hayward, W. S. *Proc. natn. Acad. Sci. U.S.A.* **24**, 7842-7846 (1982).
19. Grosveld, F. *et al. Nucleic Acids Res.* **10**, 6715-6732 (1982).
20. Erikson, J., Finan, J., Nowell, P. C. & Croce, C. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5611-5615 (1982).
21. Southern, E. J. *molec. Biol.* **98**, 503-515 (1975).
22. Saito, H., Hayward, A. C., Wiman, K., Hayward, W. S. & Tonegawa, S. *Proc. natn. Acad. Sci. U.S.A.* **80** (in the press).
23. Berk, A. & Sharp, P. A. *Cell* **12**, 721-732 (1977).
24. Weaver, R. F. & Weissman, C. *Nucleic Acids Res.* **7**, 1175-1193 (1979).
25. Colby, W. W., Chen, E. Y., Smith, D. H. & Levinson, A. D. *Nature* **301**, 722-725 (1983).
26. Goldberg, M. thesis, Stanford Univ. (1980).
27. Breathnach, R. & Chambon, P. A. *Rev. Biochem.* **50**, 349-384 (1981).
28. Proudfoot, N. & Brownlee, G. *Nature* **263**, 211-214 (1976).
29. Mulligan, R. C. & Berg, P. *Science* **209**, 1422-1427 (1980).
30. Weiher, H., Konig, M. & Gruss, P. *Science* **219**, 626-631 (1983).
31. Hayday, A. C. *et al. Cold Spring Harb. Symp. quant. Biol.* (in the press).
32. Stanton, L. W., Watt, R. & Marcu, K. M. *Nature* **303**, 401-406 (1983).
33. Benoist, C. & Charbon, P. *Nature* **290**, 304-309 (1981).
34. Nishikura, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4822-4862 (1983).
35. Calame, K., Kim, S., Lalley, P., Hill, R., Davis, M. & Hood, L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6994-6998 (1982).
36. Neuberger, M. & Calabi, F. *Nature* **305**, 240-243 (1983).
37. Erikson, J., ar-Rushdi, A., Drwina, H. L., Nowell, P. C. & Croce, C. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 820-824 (1983).
38. Cory, S. *et al. EMBO J.* **2**, 213-216 (1983).
39. Sandri-Goldin, R. M., Goldin, A. L., Levine, M. & Glorioso, J. C. *Molec. cell. Biol.* **7**, 743-752 (1981).
40. Ravetch, J. V., Siebenlist, U., Korsmeyer, S., Waldmann, T. & Leder, P. *Cell* **27**, 583-591 (1981).
41. Maxam, A. & Gilbert, S. *Meth. Enzym.* **65**, 499-560 (1980).
42. Thomas, P. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201-5205 (1980).
43. Ghosh, P. K., Reddy, V. B., Swinscoe, J., Lebowitz, P. & Weissman, S. M. *J. molec. Biol.* **126**, 813-846 (1978).
44. Gillies, S. D. & Tonegawa, S. *Nucleic Acids Res.* **11**, 7981-7997 (1983).
45. Brutlag, D. L., Clayton, J., Friedland, P. & Kedes, L. *Nucleic Acids Res.* **10**, 279-294 (1982).
46. Land, H., Parada, L. F. & Weinberg, R. A. *Nature* **304**, 576-580 (1983).

A lower limit for the mass of the compact object in SS433—a black hole?

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The SS433 system ejects two relativistic jets with a velocity of $0.26c$. The two jets are in opposite directions, precessing with a period of 160 days¹. The system is assumed to include a compact object which rotates around a normal star with a period of 13 days^{2,3}. There are strong indications for the presence of an accretion disk around the compact object⁴. Based on study of a photometric light curve obtained during 3 years of observations⁵, we suggest here that the compact object might be a black hole.

The photometric characteristics of SS433 have been studied extensively in the past few years⁴⁻¹². Despite the erratic photometric stellar behaviour, a 13 day and 160 day periodicities have been clearly detected (refs 4-12 and 5, 10-12 respectively). The averaged 13-day light curve is highly structural and varies with the 160-day period^{4,5,8-10}. However, in one third of the 160-day cycle, when the jets inclination is minimal, the averaged binary light curve is much more smooth and symmetric⁵. This light curve is reproduced in Fig. 1, where one can notice two symmetrical eclipses separated by a 'plateau', very similar to an Algol type light curve observed in many binary systems. This familiar character of the binary light curve has also been recognized by other investigators⁹.

If one assumes that Fig. 1 is an Algol-type light curve not only in form but also in essence, that is, it does indeed represent the mutual occultation of two light sources¹³, this curve can be used to estimate the mass ratio of the binary system¹⁴. We considered a system with two components: an accretion disk and a 'normal' star. These two objects are necessary parts of many models of SS433^{5,11,15-18}.

In this work we used the following assumptions: (1) The primary minimum is due to the occultation of the disk by the normal star. (2) The binary plane of motion is orthogonal to the precession axis of the jets. (3) The normal to the disk is orientated at the instantaneous jet direction. (4) The normal star fills its critical lobe. (5) The disk is planar.

Consulting Fig. 1, we further assume that: (6) The eclipse of the disk is not total. (7) The width of the disk eclipse is $0.30 \pm 0.02P$, where P is the binary period.

A computer program to calculate the relative occulted area of the disk, with a given radius R_d and orientation, has been coded, using a Roche lobe geometry [assumption (4)]. This geometry is determined by the mass ratio

$$q = M_{\text{normal}} / M_{\text{compact}}$$

where M_{normal} and M_{compact} are the masses of the normal object and the compact star, respectively.

The precessional model for the jets yields $i = 79^\circ$ and $\theta = 20^\circ$, where i is the binary inclination [assumption (2)] and θ is the precessional angle¹. We use these values for determining our line of sight and the disk orientation [assumption (3)]. For the particular 160-day phase section studied here we assume that the normal to the disk is in the plane which contains the normal to the binary orbital plane and our line of sight. Our numerical results, drawn at the q, R_d plane, are depicted in Fig. 2. Curve *a* represents the values for which the eclipse width is 0.3 of the binary period P . The dashed line above [under] curve *a* stands

for eclipse width of $0.32P$ [$0.28P$], according to our error estimate. Curve *b* of Fig. 2 represents the points in which a total eclipse is attained just at mid eclipse. For the points in the domain above curve *b*, with larger R_d the eclipse is not total. The two constraints together yield

$$q \leq 2.3 \quad (1)$$

To estimate the masses of the two components we further assume that the radial velocity curve of the He II emission line¹⁹ represents the orbital motion of the compact object. Crampton and Hutchings¹⁹ best value for the amplitude of the radial velocity curve is:

$$K = 195 \text{ km s}^{-1} \quad (2)$$

and they put a lower limit of 150 km s^{-1} for this parameter. In Fig. 3, equations (1) and (2) and the lower limit for K have been used to exclude certain domains in the $(M_{\text{compact}}, M_{\text{normal}})$ plane. We get

$$\begin{aligned} M_{\text{compact}} &\geq 4.3 M_\odot \\ M_{\text{normal}} &\geq 10 M_\odot \end{aligned} \quad (3)$$

All seven assumptions mentioned above are necessary for deriving equation (1). Consider, for example, assumptions (1)–(3). No solid proof can be found for their validity, and other competing assumptions^{20,21} cannot be excluded. However, as assumptions (1)–(3) seem to be consistent with the spectroscopic and photometric characteristics of the system, and as they are a necessary part of the 'precessing disk' model¹⁶, these assumptions are adopted here. Assumption (4), on the other hand, is not necessarily essential for all precessing disk models. However, the large mass accretion rate¹⁷, the indication for a stream^{8,19} and for a hot spot⁵ in the system, all suggest that the mass transfer is through an L_1 overflow¹⁷. This implies that the normal star fills its critical lobe^{17,18} and hence our assumption (4).

Assumption (5) is certainly only an approximation; however, our results would be modified only slightly if the disk structure is somewhere between a planar and a spherical shape.

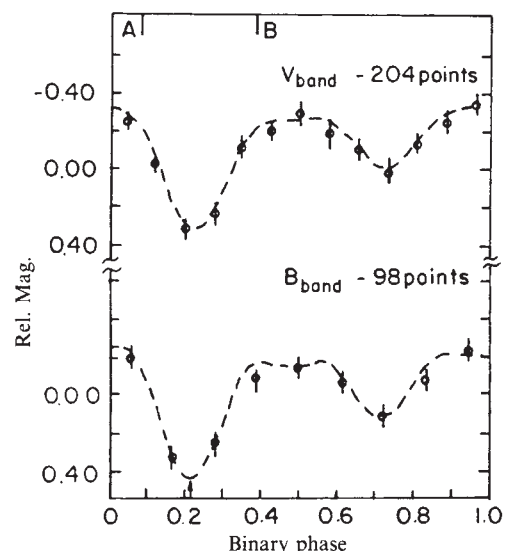


Fig. 1 A reproduction of the binary light curves in V and B bands. The plotted points are the average magnitudes in each of the corresponding bins in the phase axis. The dashed lines were obtained by a least square fitting of all the data points to a 5 term Fourier series in the basic period. The arrow indicates the phase which is a quarter of a cycle after maximum positive velocity of the He II emission lines. The phase interval ($0.3P$) between the A and B bars indicates the eclipse duration. The light curves were obtained for $0.33 \leq \Psi_{164} < 0.666$ where $\Psi_{164} = (\text{JD} - 2444068.5)/164$.

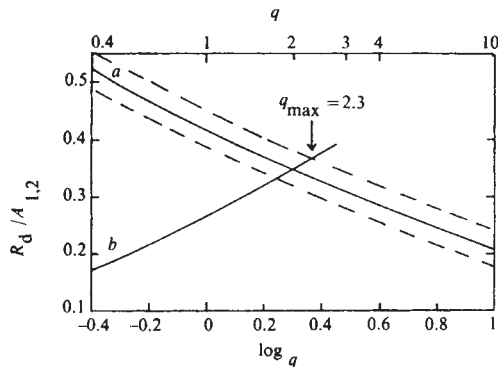


Fig. 2 The constraints on the disk radius R_d , in units of the binary separation $A_{1,2}$ as a function of q , the mass ratio.

There are several indications that the disk eclipse is not total [assumption (6)]. First, the continuous curves of Fig. 1, which have been adopted to represent the averaged light curves in V and B⁵, do not include a flat constant minimum of any length. Secondly, the HeII line appears through the whole binary period, even during mid eclipse¹⁹. It implies that the source of this emission line (centre of disk, disk's corona?) is not totally eclipsed. As the He II line is probably generated close to the centre of the disk and not at its edge, its appearance during mid eclipse is a strong evidence against the total eclipse hypothesis. Thirdly, the intensity at minimum is larger than half the total luminosity while the disk apparently contributes more than half of the total intensity in V and B⁸. All these arguments make assumption (6) very attractive. We assume, therefore, that if the disk radius is R_d , at least one point on the disk central plane at distance R_d from its center is not occulted. According to this assumption the disk thickness^{11,16} did not enter our calculations. The assumption (7) value has been deduced from Fig. 1 and the errors represent our eye estimate.

Our derived constraints on the masses [equation (3)] depend on K to the third power, therefore our conclusion is very sensitive to its exact value. Indeed, Crampton and Hutchings¹⁹ considered the possibility that their value for K does not represent the true orbital motion of the compact object. They estimate the value of 150 km s^{-1} as the lower limit for K when effects that produce spurious high amplitude are taken into account. One important effect of this kind is a partial eclipse of the disk during the phase of maximum radial velocity. The fact that the length of the disk eclipse is less than $0.32P$ significantly smaller than 0.5, excludes this effect in our case. We therefore consider the lower limit for K as real.

The two curves of Fig. 2 were obtained by using a Roche geometry, assuming corotation and alignment of the normal star. However, several works^{5,18} suggested that this might not be the case, and therefore the geometry is somewhat different^{22,23}. Consider, for example, the case in which $\Omega = \omega_{\text{stellar}}/\omega_{\text{orbital}} = 1.4$, where ω_{stellar} and ω_{orbital} are the rotational frequencies of the normal star and the orbital motion, respectively. In such a case, the upper limit for q should be 3, and the lower limit for M_{compact} would be $3M_{\odot}$. With $\Omega = 2$, the implied mass of the compact object is $1.5M_{\odot}$. The last pair of values is, however, very unlikely if one assumes that the basic precession is generated by tidal forces exerted on the normal star. Calculations by Hut and van den Heuvel¹⁸ show that with $\Omega \geq 1.4$ and $M_{\text{compact}} = 1.5M_{\odot}$, a 13d orbital period and a 160d precessional period cannot be accounted for. One can also argue that the possible non-alignment of the star is relatively small ($\sim 20^\circ$) and therefore it will modify our results only slightly²⁴. We therefore regard equations 1 and 3 as realistic limits.

An interpretation of the entire light curve of SS433 requires a detailed geometrical and physical model for the system which should include many parameters. In particular the relative intensity of the different sources in the system and the distribu-

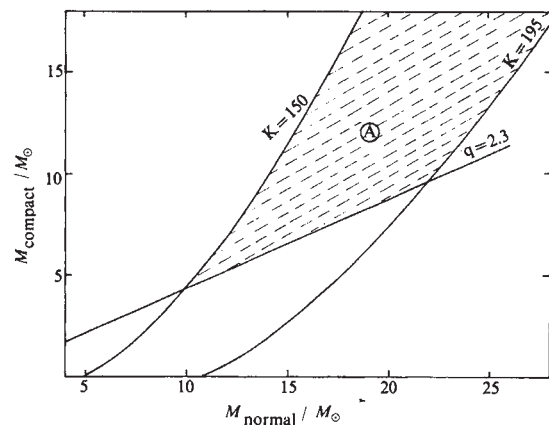


Fig. 3 The constraints on the masses of the two components. A is the allowed domain.

tion of their surface brightness should be incorporated in such a model. Indeed, such an interpretation is attempted by one of us²⁵ (E.M.L.) in a separate work, in which a theoretical synthetic light curve is fitted to all the available photometric data. For this purpose Leibowitz²⁵ assumes a hot spot as an additional source of light characterized by a very special radiation pattern. He also assumes a uniform surface brightness over the disk and neglects limb darkening of the normal star. Our approach, on the other hand, is to analyse a smooth and simple section of the light curve, without introducing any further assumptions or parameters other than those used in the most basic and common precessing disk models. Here we have considered only the disk radius and the mass ratio as free parameters. Despite the difference in approach and in the analyzing technique and despite the many more parameters in Leibowitz's model, his findings are consistent with this work.

Our basic and simple picture has yielded a most likely lower limit of $4.3 M_{\odot}$ for the compact object, (equation (3)). This limit is much larger than the mass of the most massive neutron star observed so far²⁶. It is also larger than most theoretical upper limits for this value²⁶. We therefore conclude, that it is tentatively reasonable to assume a black hole for the compact object of SS433. Further photometric and spectroscopic observations in SS433 are clearly required in order to reaffirm our conclusion and in order to improve the constraints on the mass of the compact object in this system.

We thank M. Milgrom for helpful discussions and N. Brosch, A. Fabian, D. Gies, K. Horne, J. Matese, J. Refaelli, E. van den Heuvel and D. Whitmore for many useful comments.

Received 3 August; accepted 26 October 1983.

1. Margon, B. *Science* **215**, 247–252 (1982).
2. Crampton, D., Cowley, A. P. & Hutchings, J. B. *Astrophys. J. Lett.* **235**, L131–L135 (1980).
3. Crampton, D. & Hutchings, J. B. *Vistas Astron.* **25**, 13–25 (1981).
4. Kemp, J. C., Barbour, M. S., Kemp, G. N. & Hogood, D. M. *Vistas Astron.* **25**, 31–43 (1981).
5. Leibowitz, E. M. *et al. Mon. Not. R. astr. Soc.* (in the press).
6. Kemp, J. C., Barbour, M. S., Arbabi, M., Leibowitz, E. M. & Mazeh, T. *Astrophys. J. Lett.* **238**, L133–L138 (1980).
7. Gladyshev, S. A., Kurochkin, N. E., Novikov, I. D. & Cherepashchuk, A. M. (USSR) *Astron. Tsirk.* No. 1086, 1–8 (1979).
8. Cherepashchuk, A. M. *Mon. Not. R. Astr. Soc.* **194**, 761–769 (1981).
9. Gladyshev, S. A. *Sov. Astron. Lett.* **7**, 330–333 (1981).
10. Mazeh, T., Leibowitz, E. M. & Lahav, O. *Astrophys. Letters* **22**, 185–191 (1981).
11. Anderson, S. F., Margon, B. & Grandi, S. A. *Astrophys. J.* **269**, 605–612 (1983).
12. Henson, G. D., Kemp, J. C., Barbour, M. S., Kraus, D. J., Leibowitz, E. M. & Mazeh, T. *Astrophys. J. Lett.* (in the press).
13. Russell, H. N. *Astrophys. J.* **35**, 315 (1912).
14. Whitmore, D. P. & Matese, J. J. *Mon. Not. R. Astr. Soc.* **194**, 293–299 (1981).
15. Katz, J. I. *Astrophys. J. Lett.* **236**, L127–L130 (1980).
16. Katz, J. I., Anderson, S. F., Margon, B. & Grandi, S. A. *Astrophys. J.* **260**, 780–793 (1982).
17. van den Heuvel, E. P. J., Ostriker, J. P. & Peterson, J. A. *Astron. Astrophys.* **81**, L7–L10 (1980).
18. Hut, P. & van den Heuvel, E. P. J. *Astron. Astrophys.* **94**, 327–332 (1981).
19. Crampton, D. & Hutchings, J. B. *Astrophys. J.* **251**, 604–610 (1981).
20. Kundt, W. *Nature* **282**, 52–53 (1979).
21. Kundt, W. *Vistas Astron.* **25**, 153–164 (1981).
22. Pratt, J. P. & Strittmatter, P. A. *Astrophys. J. Lett.* **204**, L29–L33 (1976).
23. Bahcall, J. H. *A. Rev. Astr. Astrophys.* **16**, 241–264 (1978).
24. Avni, Y. & Schiller, N. *Astrophys. J.* **257**, 703–714 (1982).
25. Leibowitz, E. M. *Mon. Not. R. astr. Soc.* (submitted for publication).
26. Joss, P. C. & Rappaport, S. A. *Neutron Stars in Interacting Binary Systems* CSR-AT-82-10 (1982).

Continuing formation of the central star cluster in M87

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The rising velocity dispersion of the stars in the neighbourhood of the central luminosity spike observed¹⁻³ in the elliptical radio galaxy M87 was originally considered to be evidence for the presence of a massive black hole⁴ ($M \sim 5 \times 10^9 M_\odot$). A later spectrum⁵ taken in good seeing, however, shows that most of the light in the spike is due to stars, which restricts any black hole model; the luminosity spike may be a star cluster formed from gas infalling from the outer regions of M87^{5,6}. Our analysis⁷ of Einstein Observatory X-ray data on M87 revealed a cooling inflow of gas occurring at the rate required to build such a cluster. We show here that the back-reaction on the flow of the nuclear luminosity is small unless M87 was much brighter in the past and we suggest that massive central star clusters may be common in the central galaxies in many clusters and, on a smaller scale, in many elliptical galaxies.

X-ray continuum^{5,8} and emission line^{5,9,10} measurements of the temperature of the gas in M87 have established the presence of a temperature gradient which implies that the gas is cooling and accreting onto the galaxy¹¹⁻¹⁴. We have combined these spectral and imaging¹⁵ data to determine in detail the gas temperature and density profiles and thence the mass flow rate, $\dot{M}$, at each radius⁷. $\dot{M}$ drops from $10 M_\odot \text{ yr}^{-1}$ beyond about 50 kpc to $\sim 1 M_\odot \text{ yr}^{-1}$ at 750 pc, or 10 arc s, from the nucleus. At this inner radius, which is determined by the quality of the data, the gas temperature, T , is $\sim 10^7$ K. It must fall steeply at some point within this region, liberating an X-ray luminosity $\sim 5/2 \dot{M} / \mu m_{\text{H}} kT$, where μm_{H} is the mean mass per particle. We have identified this luminosity with the 2-3 arc s extended X-ray source¹⁶ that coincides with the central luminosity spike. Gas is thus cooling and collapsing there at a rate of $\sim 1 M_\odot \text{ yr}^{-1}$ and we presume that most of it forms stars. Up to $\sim 10^{10} M_\odot$ is added over the age of the galaxy if the flow is steady. The high pressure in a cooling flow ($\sim 10^6 \text{ cm}^{-3}$ K) leads to the formation of low mass stars^{17,18} and we therefore expect the star cluster so formed to have a large ratio of mass to luminosity ($M/L > 10$). The optical colours¹ are consistent with the models of Sarazin and O'Connell¹⁸.

The optical jet in M87 is evidence for activity in the nucleus and we therefore consider the possible influence of nuclear luminosity on the cooling flow. The flow itself would provide sufficient matter to power even a bright quasar if it were accreted by a compact object. The ionization equilibrium of gas in a cooling flow was calculated numerically by iterating the effects of photo-absorption and collisional ionization due to X-rays from the gas as well as from a nuclear X-ray source ΔL . The important bound-bound, bound-free and free-free radiative processes above 10^6 K of H, C, N, O, S, Si and Fe were included in the calculations. The density and temperature of successive radial shells of gas were interpolated from our solution⁷ and extrapolated into the centre assuming constant pressure and $\dot{M}$. The results below are quoted for a radius of 190 pc, which is within the core of M87.

Departures from collisional equilibrium alone are small (less than a factor of 2) for the dominant ions of C, N, O and Fe provided that the $\Delta L_{41} < 10$, where $\Delta L = \Delta L_{41} \times 10^{41} \text{ erg s}^{-1}$ in the 0.5-4.5 keV range (an energy index of 0.75 was used). Radiation pressure is negligible in any reasonable distribution of mass in the core of M87 provided that $\Delta L_{41} < 10^5$. The local heating rate due to photoabsorption and to Compton interactions is found to exceed the local cooling rate in our calculations when $\Delta L_{41} > 10^2$. The cooling flow should be strongly disrupted above that luminosity. The observations¹⁷ indicate $\Delta L_{41} < 4$, which means that the core of M87 must brighten considerably before the cooling flow is stopped. (Increasing $\dot{M}$ does, of course, increase the limits on ΔL .) The smooth X-ray surface brightness profile is observational evidence that the flow has lasted for times comparable with a typical cooling time of $\sim 10^9$ yr.

Cooling flows are relatively common in clusters surrounding a single cD galaxy^{19,20} and we may expect that some of the inferred $5\text{--}500 M_\odot \text{ yr}^{-1}$ of accreting gas forms central star clusters in them. We have re-analysed the X-ray surface brightness profile²¹ of NGC1275 in the Perseus cluster to determine $\dot{M}(r)$ within a reasonable gravitational potential. Once again, we find that $\dot{M}$ increases with radius, r , up to $400 M_\odot \text{ yr}^{-1}$ at 100 kpc and that $\sim 40 M_\odot \text{ yr}^{-1}$ passes into the inner 10 kpc (our best resolution) of the galaxy. It appears that the stars form over a large range of radii, comparable with the extent of the optical filaments²², and by analogy with M87, it is likely that NGC1275 contains a large star cluster in its core. Scaled-down versions of the flows in M87 and NGC1275 may occur in many elliptical galaxies²³ and could also take place in the bulges of spiral galaxies. The centre of M31 may possibly contain a cluster of low mass stars (ref. 24; but see 25). The evolution of the densest core of a large central star cluster may in turn be related to the nuclear activity seen in many galaxies. Gravitational accretion by any central mass takes place from a hot ($10^6\text{--}10^7$ K) medium.

We thank R. Terlevich for discussion and S. Inagaki for a preprint. A.C.F. acknowledges support from the Royal Society.

Received 4 August; accepted 2 November 1983.

1. Young *et al.* *Astrophys. J.* **221**, 721-730 (1978).
2. de Vaucouleurs, G. & Nieto, J.-L. *Astrophys. J.* **230**, 697 (1979).
3. Dressler, A. *Astrophys. J. Lett.* **240**, L11-L16 (1980).
4. Sargent *et al.* *Astrophys. J.* **221**, 731-744 (1978).
5. Lea, S. M., Mushotzky, R. F. & Holt, S. S. *Astrophys. J.* **262**, 24-32 (1982).
6. Okazaki, A. T. & Inagaki, S. *Publ. astr. Soc. Japan* (submitted).
7. Stewart, G. C., Canizares, C. R., Fabian, A. C. & Nulsen, P. E. J. *Astrophys. J.* (in the press).
8. Fabricant, D. & Gorenstein, P. *Astrophys. J.* **267**, 535-546 (1983).
9. Canizares *et al.* *Astrophys. J. Lett.* **234**, L33-L38 (1979).
10. Canizares, C. R., Clark, G. W., Jernigan, J. G. & Markert, T. H. *Astrophys. J. Lett.* **262**, L33-L43 (1982).
11. Cowie, L. L. & Binney, J. *Astrophys. J.* **215**, 723-732 (1977).
12. Fabian, A. C. & Nulsen, P. E. J. *Mon. Not. R. astr. Soc.* **180**, 479-484 (1977).

13. Matthews, W. G. & Bregman, J. N. *Astrophys. J.* **224**, 308-319 (1978).
14. Binney, J. & Cowie, L. L. *Astrophys. J.* **247**, 464-472 (1981).
15. Fabricant, D., Lecar, M. & Gorenstein, P. *Astrophys. J.* **241**, 552-560 (1978).
16. Schreier, E. J., Gorenstein, P. & Feigelson, E. D. *Astrophys. J.* **261**, 42-50 (1982).
17. Fabian, A. C., Nulsen, P. E. J. & Canizares, C. R. *Mon. Not. R. astr. Soc.* **201**, 933-938 (1982).
18. Sarazin, C. L. & O'Connell, R. W. *Astrophys. J.* **268**, 552-560 (1983).
19. Stewart, G. C., Fabian, A. C., Jones, C. & Forman, W. *Astrophys. J.* (submitted).
20. Stewart, G. C., Canizares, C. R. & Fabian, A. C. *Astrophys. J.* **272**, 449-455 (1983).
21. Fabian, A. C., Hu, E. M., Cowie, L. L. & Grindlay, J. *Astrophys. J.* **248**, 47-54 (1981).
22. Lynds, R. *Astrophys. J. Lett.* **159**, L151-L154 (1970).
23. Nulsen *et al.* *Mon. Not. R. astr. Soc.* (in the press).
24. Faber, S. M. & French, H. B. *Astrophys. J.* **235**, 405-412 (1980).
25. Persson *et al.* *Astrophys. J.* **240**, 779 (1980).

Mystery cloud of AD 536

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Dry fogs appear in the atmosphere when large volcanic eruptions inject massive quantities of fine silicate ash and aerosol-forming sulphur gases into the troposphere and stratosphere. Although the ash gravitationally settles out within weeks, the aerosols spread around the globe and can remain suspended in the stratosphere for years. Because solar radiation is easily absorbed and backscattered by the volcanic particles, a haziness in the sky and a dimming of the Sun and Moon are produced. Very dense and widespread dry fogs occur, on the average, once every few centuries¹⁻³. The sizes and intensities of some of the largest of them before the modern scientific era have been estimated by several indirect methods^{2,4,5}. The densest and most persistent dry fog on record was observed in Europe and the Middle East during AD 536 and 537. Despite the earliness of the date, there is sufficient detailed information to estimate the optical depth and mass of this remarkable stratospheric dust cloud. The importance of this cloud resides in the fact that its mass and its climatic consequences appear to exceed those of any other volcanic cloud observed during the past three millennia. Although the volcano responsible remains a mystery, a tropical location (perhaps the volcano Rabaul on the island of New Britain, Papua New Guinea) can be tentatively inferred.

Four reliable contemporary descriptions of the sky conditions in AD 536–537 exist⁶. Procopius⁷, a Byzantine historian who lived in Rome (42°N) at the time, wrote that “the Sun gave forth its light without brightness, like the Moon, during this whole year, and it seemed exceedingly like the Sun in eclipse, for the beams it shed were not clear nor such as it is accustomed to shed . . . and it was the time when Justinian was in the tenth year of his reign”. From Constantinople (41°N) Lydus⁸ observed that “the Sun became dim in the course of the recently passed fourteenth indiction, for nearly a whole year, when Belisarius had the highest honour, so that the fruits were killed at an unseasonable time”. An anonymous chronicler (associated by tradition with the name of Zacharias of Mytilene⁹) also referred to observations made from Constantinople in the following terms: “in the year fourteen . . . the Sun began to be darkened by day and the Moon by night . . . from the 24th of March in this year till the 24th of June in the following year fifteen”. According to another contemporary writer (probably John of Ephesus), describing conditions in Mesopotamia (30–37°N) and later quoted by Michael the Syrian¹⁰ and Bar-Hebraeus¹¹: “in the year 848 of the Greeks . . . the Sun was dark and its darkness lasted for eighteen months; each day it shone for about four hours, and still this light was only a feeble shadow . . . the fruits did not ripen and the wine tasted like sour grapes”.

What can be made of such astonishing reports? In ordinary conditions, the Sun's radiance would appear perceptibly diminished only if the Sun lay less than $\sim 2.5^\circ$ in apparent altitude above the horizon¹². The normal visual extinction at apparent zenith angle $z = 87.5^\circ$ is 3.4 mag (astronomical magnitudes)¹³. If the Sun shone for only 4 hours a day at a latitude of 33° N during the fruit-growing season (spring and summer) of AD 536, then it must have begun setting at $z \sim 30^\circ$. The total visual extinction produced by the atmosphere in combination with the volcanic dust can be adequately represented by Bouguer's law¹⁴; in the present case

$$[(\Delta m)_0^D + 0.20] \sec z = 3.4$$

where $(\Delta m)_0^D$ is the zenithal volcanic dust contribution and 0.20 mag refers to the unperturbed atmosphere. It follows that

$(\Delta m)_0^D \sim 2.7$ mag, corresponding to an excess optical depth $\tau_D = 0.921$ ($(\Delta m)_0^D \approx 2.5$). At maximum altitude both the Sun and the full Moon would have appeared ~ 10 times fainter than normal, which can readily explain their reported ‘darkening’. Scattered sunlight, however, would still have illuminated the rest of the sky, which therefore would not have appeared as dark. Although the derived optical depth excess is rather uncertain, it can be checked below by an independent method.

What sort of volcanic eruption could have produced such a dense dust veil? Not a Mediterranean eruption⁶, which would almost certainly have been mentioned by Procopius. A clue is found in the duration of the dry fog. Historically, dry fogs have always developed rather quickly, although the dates of their slow disappearances are susceptible to visual impression and have inevitably been uncertain by a few months. The dry fog of AD 536 first appeared in the Mediterranean region in late March. According to the observers at $41\text{--}42^\circ$ N, it lasted 12–15 months, that is, until March–June AD 537; this period agrees in an acceptable way with the variously reported dates. At $30\text{--}37^\circ$ N, however, the duration of the dry fog was 18 months. Its prolonged visibility at the more southerly latitudes suggests that the haze was significantly thicker there, and hence that the eruption which produced the aerosols was situated somewhere to the south. If so, the location was probably in the tropics (23° S– 23° N) because aerosols from eruptions south of 23° S do not penetrate the Northern Hemisphere in significant amounts. Only 2 to 3 weeks would be needed for the stratospheric aerosols to reach Mediterranean latitudes. A large volcanic eruption that may be consistent with the year, March season, and possible tropical latitude of the AD 536 event was the explosion of Rabaul (4° S) on the island of New Britain, off New Guinea, which has been radiocarbon-dated to AD 540 ± 90 (ref. 15). This eruption dispersed at least 11 km^3 (bulk volume) of near-source pumice and ash, mostly to the west¹⁶, which suggests that the March–October southeasterly monsoon¹⁷ was blowing at the time. (Most of the distal ash would have fallen into the Bismarck Sea, and could greatly exceed in volume the near-source ejecta.)

A major tropical eruption in AD 536 would also be expected to deposit sulphuric acid aerosols on the polar ice caps, where they would remain preserved in compacted annual snow layers. A very high sulphuric acid signal has in fact been detected in a Greenland ice core by Hammer *et al.*⁵, who assigned a date of AD 540 ± 10 , and in another Greenland ice core by Herron¹⁸, who estimated $\sim$ AD 535. There is little doubt that the acidity signal refers to the same event that produced the dry fog of AD 536, because both kinds of volcanic signal at the exceptionally high levels discussed here occur only once every few centuries on the average. The strength of this ancient volcanic signal relative to the signal obtained from the more recent eruption of another tropical volcano, Tambora (which exploded in April 1815), can be estimated as follows. Herron's data imply relative signal strengths (after subtraction of background) of 0.63:1.00 for the AD 536 eruption and the Eldgjá (Iceland, $\sim$ AD 934) eruption, while the data of Hammer *et al.*⁵ (who did not provide the signal strength for the AD 536 eruption) imply a ratio of 1.00:0.37 for the Eldgjá and Tambora eruptions. It follows that the AD 536 signal exceeds that of Tambora by a factor of 1.7. As the eruption of Tambora increased the stratospheric optical depth at north-temperate latitudes in 1815 by $\tau_D = 1.3$ as derived by several methods (ref. 19), the excess stratospheric turbidity at these latitudes in AD 536 must have been $\tau_D = 1.3 \times 1.7 = 2.2$. (If Krakatoa, a tropical volcano that erupted in 1883, is used as the standard for comparison, essentially the same result is obtained.) This is consistent with the preceding result based on the ancient extinction reports, and therefore supports the idea that the eruption was a tropical one because the strength of the ice-core acidity signal depends on eruption latitude⁵ and no correction for latitude has been applied here. Peak global aerosol loading of the stratosphere can be computed from the formula $M_D = 1.5 \times 10^{14} \tau_D \text{ g}$, which is insensitive to the adopted modal particle radius r if $0.15 \leq r \leq$

0.7 μm —certainly the case for modern volcanic eruptions (ref. 19). The loading in AD 536 was therefore $\sim 3 \times 10^{14}$ g.

The AD 536 stratospheric 'mystery cloud' presumably decayed exponentially with a typical e -folding time of ~ 1 yr (ref. 19). If so, its optical depth would have dropped to less than 0.1 by November and December AD 539, when a faint cometary tail was visually sighted from Europe⁷ and China²⁰.

Climatologically, the winter of AD 536–537 in the Middle East was an unusually severe one, attended by extraordinary snowfalls in Mesopotamia^{9,21}. During the preceding summer (and possibly the following one as well), the fruits, including grapes, did not properly ripen in at least some parts of the Middle East and Europe. Historically, cold weather and poor harvests of grapes and other crops have tended to follow very large explosive volcanic eruptions². The accepted reason is that volcanic dust clouds screen some of the incoming sunlight and thereby cause a reduction of the Earth's average surface temperature. Although it seems that Tambora's large eruption did not catastrophically affect the Middle Eastern growing season²², the aerosol-producing eruption of AD 536, which was twice as large, apparently did.

I thank M. R. Rampino for useful comments.

Received 22 August; accepted 10 November 1983.

1. Russell, F. A. R. *The Eruption of Krakatoa and Subsequent Phenomena* (ed. Symons, G. J.) 384–405 (Truebner, London, 1888).
2. Lamb, H. H. *Phil. Trans. R. Soc. A* **266**, 425–533 (1970).
3. Stothers, R. B. & Rampino, M. R. *Science* **222**, 411–413 (1983).
4. Deirmendjian, D. *Adv. Geophys.* **16**, 267–296 (1973).
5. Hammer, C. U., Clausen, H. B. & Dansgaard, W. *Nature* **288**, 230–235 (1980).
6. Stothers, R. B. & Rampino, M. R. *J. geophys. Res.* **88**, 6357–6371 (1983).
7. Procopius *History of the Wars* (transl. Dewing, B. H.) 2.4.1–2, 4.14.5–6 (Harvard University Press, Cambridge, Massachusetts, 1916).
8. Lydus, J. L. *On Portents* (ed. Wachsmuth, C.) 9c (Teubner, Leipzig, 1863).
9. Zacharias of Mytilene *Chronicle* (transl. Hamilton, F. J. & Brooks, E. W.) 9.19, 10.1 (Methuen, London, 1899).
10. Michael the Syrian *Chronicle* (transl. (into French) Chabot, J.-B.) 9.296 (Belles-Lettres, Paris, 1901).
11. Bar-Hebraeus *Chronography* (transl. Budge, E. A. W.) 8.79–80 (Oxford University Press, London, 1932).
12. Minnaert, M. *The Nature of Light and Colour in the Open Air* (transl. Kremer-Priest, H. M. & Jay, K. E. B.) 55, 269 (Dover, New York, 1954).
13. Allen, C. W. *Astrophysical Quantities*, 125–127 (Athlone, London, 1973).
14. Blanco, V. M. & McCuskey, S. W. *Basic Physics of the Solar System*, 93 (Addison-Wesley, Reading, Massachusetts, 1961).
15. Heming, R. F. *Bull. geol. Soc. Am.* **85**, 1253–1264 (1974).
16. Walker, G. P. L., Heming, R. F., Sprod, T. J. & Walker, H. R. *Geol. Surv. Papua New Guinea Mem.* **10**, 181–193 (1981).
17. Seltzer, L. E. (ed.) *The Columbia Lippincott Gazetteer of the World*, 1309 (Columbia University Press, New York, 1952).
18. Herron, M. M. *J. geophys. Res.* **87**, 3052–3060 (1982).
19. Stothers, R. B. *Science* (in the press).
20. Ho Peng Yoke *Vistas Astr.* **5**, 127–225 (1962).
21. John of Ephesus *Ecclesiastical History* (transl. (into Latin) van Douwen, W. J. & Land, J. P. N.) 297–298 (Muller, Amsterdam, 1889).
22. Post, J. D. *The Last Great Subsistence Crisis in the Western World*, 55–59 (Johns Hopkins University Press, Baltimore, Maryland, 1977).

Scattered UV solar radiation within the clouds of Venus

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The spectral albedo curve of Venus shows a steep decline at wavelengths between 0.5 and 0.3 μm (refs 1–3). This is of interest since absorption of solar energy in this spectral region has a strong influence on the thermal balance of the venusian atmosphere. The nature and altitude distribution of the UV absorber have not been determined, although some candidate gases have been proposed. We report here the results of measurements carried out on the last Soviet venusian descent probe Venera-14 which suggest that the UV absorption is by aerosols above an altitude of 57 km and by gases below this level.

It has long been known that some UV absorber(s) is present in the venusian clouds. The same absorbing material seems to

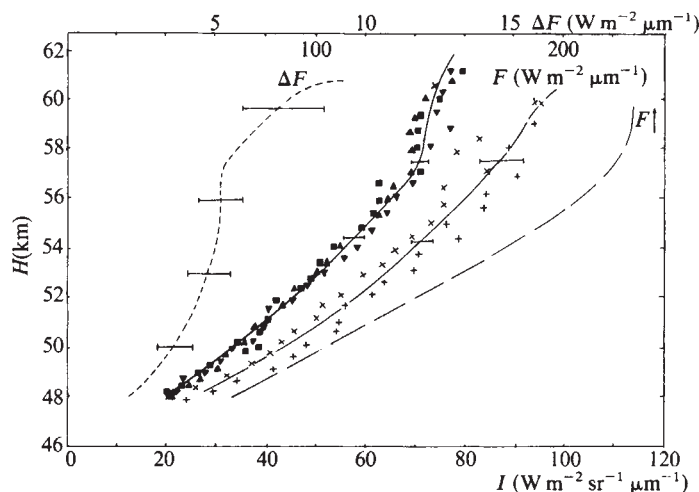


Fig. 1 Observed UV intensities at the zenith angles of 45° (x, +), 133° (v, Δ), 164° (■) as a function of altitude as well as least square fits. Also shown are the upward flux (—) and the net flux (---) densities, calculated from experimental data.

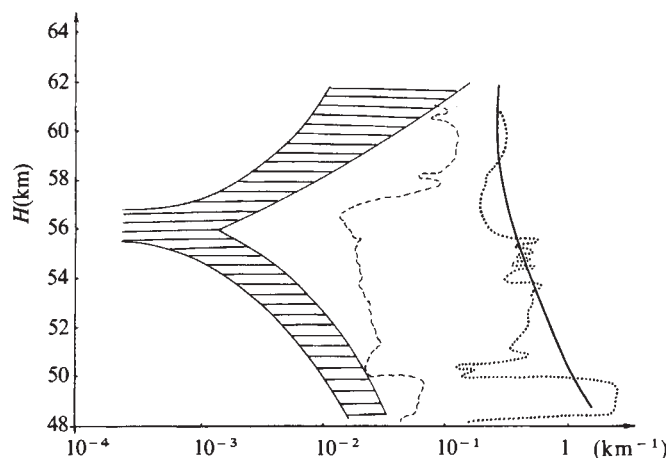


Fig. 2 Observational range in volume absorption coefficient (shaded area) and volume extinction coefficient multiplied by $(1-g)$ (—) as a result of direct computation from the UV photometer data. Also shown are the extinction coefficients multiplied by $(1-g) = 0.2$ (after ref. 13) for mode-1 only (---) and total (····).

be responsible for both the low UV albedo and for producing the UV features on Venus. Gaseous sulphur dioxide is the obvious candidate for the absorber at wavelengths shorter than 0.32 μm (ref. 4), but the nature of the substance responsible for absorption longwards of 0.32 μm is not known. Remote sensing techniques have failed to solve this problem. Pioneer Venus results^{5,6} and results from Veneras 9 and 10 (ref. 7) showed that the absorption of sunlight in the visible took place mainly in the upper cloud, which is why one of the principal goals of the Soviet Venera-14 mission was to investigate the distribution of the solar near-UV radiation within the main clouds during the descent of the probe through the atmosphere of Venus on 3 March 1982.

The UV photometer aboard Venera-14 was part of a complex optical instrument with a common angle-scanning device for the near-UV and the visible regions. The detector Ga-P/filter combination provided a bandpass of 0.06 μm at half-maximum centred at 0.37 μm with no appreciable leak beyond bandpass. The photometer had a circular field of view (FOV) with width 10° (FWHM). The instrument scanned a range of zenith angles by rotating the axis of the FOV in a vertical plane with a period of 2.5 s. (The photometer and its operating conditions are described in more detail elsewhere^{8,9}.)

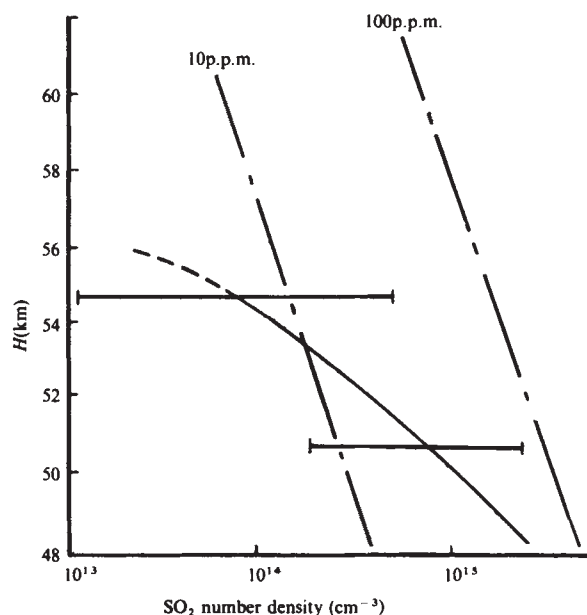


Fig. 3 Sulphur dioxide number density on the basis of the observed data on UV absorption (solid line with bars). Curves of constant mixing ratio are also shown.

The raw data from our photometric measurements as well as the results from the primary data processing are presented in Fig. 1. The data are plotted as a function of altitude (H) above the 6,052 km level. Fits by spline technique were used for least-squares approximation of the data. We define I_{45} , I_{133} , I_{164} as the solar near-UV intensities sampled at zenith angles of 45° , 133° , 164° . $F\downarrow$, $F\uparrow$, ΔF are the downward, upward and net fluxes respectively derived from intensities using the approximation:

$$I(\phi) = I(1)[a + \phi(1-a)]$$

where $I(\phi)$ denotes the angular intensity distribution, ϕ is the cosine of the zenith angle, a is the parameter derived from the least squares fit, $I(1)$ is the downwards intensity. It is a simple matter to show that:

$$F\downarrow = 2\pi I(1) \left(\frac{a}{2} + \frac{1-3}{3} \right)$$

$$F\uparrow = 2\pi I(1) \left(\frac{a}{2} - \frac{1-a}{3} \right)$$

$$\Delta F = \approx 3(I_{45} - I_{133})$$

The data set in the uppermost portion of the profile (from 62 to 59 km) is characterized by high variability of downward intensities although the upward ones are quite stable. There was no instability of downward intensities below 59 km. Inhomogeneities 1–5 km in length are expected above 59 km which does not seem to be consistent with results obtained from remote sensing orbiters which have failed to discover inhomogeneity at scales of less than 10 km (ref. 10). The above-mentioned phenomenon may resemble the 'silver lining effect'—where the photometer views the illuminated edges of the clouds at horizontally-inhomogeneous upper layers. From about 59 km down to 48 km, the downward flux decreases gradually from $1/5$ th of the incident solar radiation flux at the top of the venusian atmosphere to $1/35$ th of this value.

Using ground-based data on the UV albedo of Venus¹¹ in conjunction with results from our measurements, an effective optical depth above 60 km can be estimated as $\tau_0 = \tau(1-g) \approx 3$ where τ is the optical depth, and g is the asymmetry factor of the phase function. Consequently a thick-atmosphere approxi-

mation can be obtained and all calculations may be substantially simplified. For example, altitude profiles of the volume absorption coefficient K and extinction coefficient α of the atmosphere may be derived from $(I_{45} - I_{133})$ and $(I_{45} + I_{133})$ altitude profiles¹². The results of such an evaluation are presented in Fig. 2. Note, however, that K is extremely sensitive to computational errors which leads to some uncertainty over its value.

The altitude profile of the net flux shows that the true absorption of UV radiation is present everywhere within the cloud structure but most strongly in the upper clouds ($H > 58$ km). The next lowest layer ($58 > H > 55$ km) has the minimum UV volume absorption coefficient. These results are qualitatively consistent with the data Tomasko⁶ obtained in the visible region. In the altitude interval between 55 and 48 km the absorption is probably coupled with the increasing gas density.

Figure 2 also provides a comparison between our results and the extinction coefficient from LCPS (cloud particle size spectrometer) measurements¹³. The profiles of K and $\alpha(1-g)$ presented in Fig. 2 lead to the following conclusions:

(1) There are two different absorbers which dominate above and below the 57-km level. This is supported by different altitude dependences above and below this level and the minimum-volume absorption coefficient K observed there.

(2) The most important absorber above 57 km is probably particulate matter. Particles of mode 1 as defined by Knollenberg and Hunten¹³ can be identified as the absorbers near the cloud top. The sharp rise of absorption is crudely consistent with the rise in the mode-1 particle concentration above 57 km. Of course, the possibility cannot be ruled out that some absorbing gas has the same type of altitude distribution (due, for example, to some photochemical processes) but this seems unlikely. If mode-1 particles are responsible for the measured K value it is possible by combining Knollenberg and Hunten's data with ours to evaluate κ , the imaginary part of the refractive index. We find that $7 \times 10^{-4} < \kappa < 7 \times 10^{-3}$. The existing information is not yet sufficient for identification but now two additional limitations on the absorber are implied: the value of κ and the altitude distribution. Sulphur^{4,14}, $\text{FeCl}_3 \cdot \text{H}_2\text{SO}_4$ (ref. 15) and $(\text{NH}_4)(\text{S}_2\text{O}_3)$ (ref. 16) have been considered recently as possible constituents. Toon *et al.*¹⁴ have suggested that even a few per cent of strongly absorbing short-chain sulphur allotropes as particles, such as S_3 (absorption band centred near 4,000 Å) and S_4 (absorption band centred near 5,300 Å) could account for the solar energy deposition profile in the upper clouds at wavelengths longer than 0.32 μm . Our data seem to be consistent with an approximate 1–10% mass of sulphur within the upper clouds. A steep decline of absorption from the beginning of our measurements at 62 km to the minimum absorption detected at 56 km resembles a steep decline from the peak sulphur mass fraction at 58 km calculated by Toon¹⁴ to the small amount present below 56 km. The peak value itself was not detected in our measurements but it could be masked by a mode-1 concentration growth above 58 km.

(3) Probably the most important absorber below 57 km is a gas. The basis for this proposal is the increase of K with decreasing altitude (K increases even faster than does the atmospheric number density). An attractive candidate for this gas is SO_2 which was discovered long ago as a small constituent of the venusian low atmosphere^{17,18}, whose concentration extrapolated to the altitude ~ 50 km is about what is needed to provide the observed UV absorption. Figure 3 shows a profile of SO_2 in the altitude interval 50–57 km calculated on the basis of the hypothesis that SO_2 gas is responsible for absorption.

We have used the laboratory cross-section of SO_2 taken from refs 19, 20, although our neglect of the influence of the unresolved line structure of SO_2 bands in the radiative transfer treatment²¹ may necessitate a later re-evaluation of the SO_2 concentration. More details concerning results described above and their interpretation are given in ref. 9.

Received 28 July; accepted 3 November 1983.

1. Irvine, W. M. *J. Atmos. Sci.* **25**, 610 (1963).

2. Barker, E. S. *et al. J. Atmos. Sci.* **32**, 1205 (1975).

3. Moroz, V. I. *et al. Pis'ma Astr. Zh.* **8**, 404 (1982).
4. Esposito, L. W., Winick, J. R. & Stewart, A. I. *Geophys. Res. Lett.* **6**, 601 (1979).
5. Pollack, J. B. *et al. J. geophys. Res.* **85**, 8141 (1980).
6. Tomasko, M. G. *et al. J. geophys. Res.* **85**, 8141 (1980).
7. Ekonomov, A. P. *et al. Kosm. Issled.* **16**, 901 (1978).
8. Moshkin, B. E. *et al. Kosm. Issled.* **21**, 236 (1983).
9. Ekonomov, A. P. *et al. Kosm. Issled.* **21**, 254 (1983).
10. Rossow, W. B. *et al. J. geophys. Res.* **85**, 8107 (1980).
11. Moroz, V. I. in *Venus*, 27 (The University of Arizona Press, 1983).
12. Ustinov, E. A. *Kosm. Issled.* **15**, 768 (1977).
13. Knollenberg, R. G. & Hunten, D. M. *J. geophys. Res.* **85**, 8039 (1980).
14. Toon, O. B., Turco, R. P. & Pollack, J. P. *Icarus* **51**, 358 (1982).
15. Zasova, L. V., Krasnopolsky, V. A. & Moroz, V. I. *Adv. Space Res.* **1**, 13 (1981).
16. Titov, D. V. *Kosm. Issled.* **19**, 794 (1981).
17. Gel'man, B. G. *et al. Kosm. Issled.* **17**, 708 (1979).
18. Hoffman, J. H., Oyama, V. I. & von Zahn, U. *J. geophys. Res.* **85**, 7871 (1980).
19. Vikesland, J. P. & Stricker, S. J. *J. chem. Phys.* **60**, 660 (1974).
20. Sidebottom, H. W. *et al. J. Am. chem. Soc.* **93**, 2587 (1971).
21. Belton, M. J. S. *Icarus* **52**, 149 (1982).

Analysis for rare earth elements in accessory minerals by specimen isolated secondary ion mass spectrometry

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The distribution of rare earth elements (REEs) in minerals and mineral assemblages is of considerable geochemical significance. However, because of their low levels in most minerals, analysis for REEs is a considerable challenge. The application of secondary ion mass spectrometry (SIMS) to this problem has been limited by the presence of molecular ion interferences in the secondary ion mass spectrum. We describe here the application of the specimen isolation technique¹ to rare earth analysis by SIMS. The method effectively suppresses molecular ions while maintaining sufficient sensitivity to examine REE distribution in a variety of accessory minerals.

REEs have been used to help interpret the genesis of igneous rocks², metamorphic rocks³ and sedimentary rocks⁴, as well as the history of the Earth⁵. Their chemistry is predominantly ionic, their properties being controlled by their size. Atomic and ionic radii decrease systematically from lanthanum to lutetium as a result of increasing nuclear charge and imperfect shielding of the 4f electrons. Consequently the properties of these species, including their thermodynamic properties and their concentrations in minerals and rocks, vary sympathetically with changes in ionic radii. Only europium shows variable valency in geological environments and may be dominantly divalent or trivalent, depending on the partial pressure of oxygen. The systematic behaviour of the trivalent lanthanides together with the anomalous behaviour of Eu^{2+} , make them a very powerful tool in geochemistry. However, there are few studies that document the concentration of REEs in the suite of minerals composing a rock⁶.

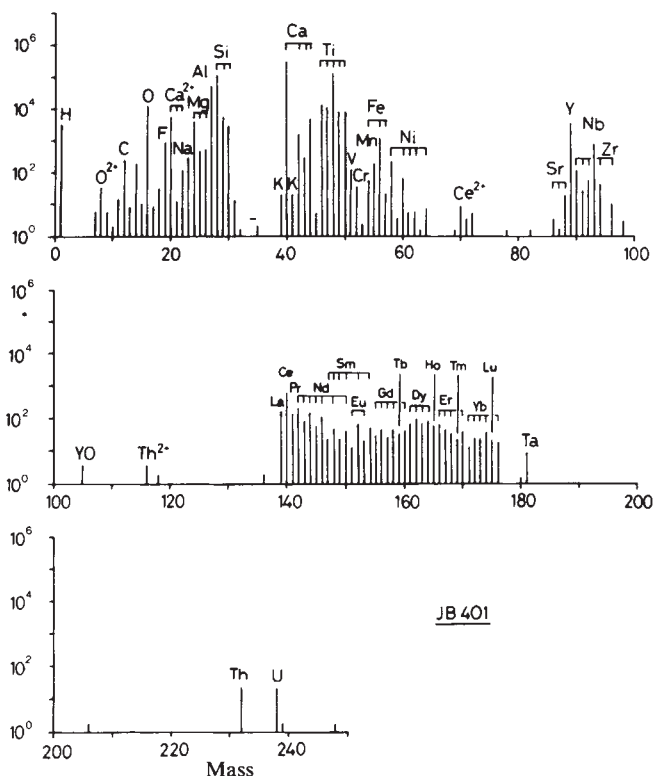


Fig. 1 Secondary ion mass spectrum of a sphene from Cardiff Mine, Ontario, Canada. Count time of 1 s over each integral m/e value.

SIMS has been recognized for some time as the potential 'ultimate weapon' for geochemical analysis⁷, however the two major stumbling blocks in realizing this potential have been resolving elemental ions from molecular ions⁸, and the inability to quantify secondary ion yields (J.B.M., H.W.N. and G.M.B., in preparation).

There are currently two approaches to removing molecular ion interferences from SIMS spectra. The first involves resolving peaks at the same unit m/e using the small mass difference between elemental and molecular peaks⁷⁻⁹. The alternative approach exploits the difference in kinetic energy distribution between molecular and elemental ions to suppress the intensity of the molecular ions. This is possible only with instruments that use a double focusing secondary column geometry such as the Nier design used in the Cameca IMS 3f. Shimizu *et al.*^{8,10,11} have refined this technique and shown it to be superior to high mass resolution in a number of applications, particularly at $m/e > 70$.

As discussed recently by Reed^{12,13}, in the case of the REEs, very high resolution is required to separate the oxide and elemental peaks (for example, a $m/\Delta m > 8,000$ is required to separate $^{169}\text{Tm}^+$ from $^{153}\text{Eu}^{16}\text{O}^+$) and the associated intensity loss is prohibitive. Kinetic energy selection fares little better as

Table 1 Observed and natural abundances¹⁷ for rare earth isotopes in Arendal sphene (a), Bear Lake sphene (b)

Nd			Sm			Gd			Yb		
Mass	Observed	Natural	Mass	Observed	Natural	Mass	Observed	Natural	Mass	Observed	Natural
	a	b		a	b		a	b		a	b
142*		27.11	144*		3.09	154*		2.15	170*		3.03
143	12.8	12.8	147	15.4	15.0	155	14.0	18.0	171	14.7	13.5
144*		23.85	148*		11.2	156	20.7	22.7	172	22.3	26.6
145	8.44	8.23	149	13.1	14.6	157	16.2	15.3	173	14.8	19.1
146	16.8	17.0	150*		7.44	158	24.8	19.9	174	31.9	24.8
148*		5.73	152	26.4	25.3	160*		21.9	176*		12.7
150*		5.62	154*		22.7						

* Peaks at these m/e values consist of an isotope from more than one element.

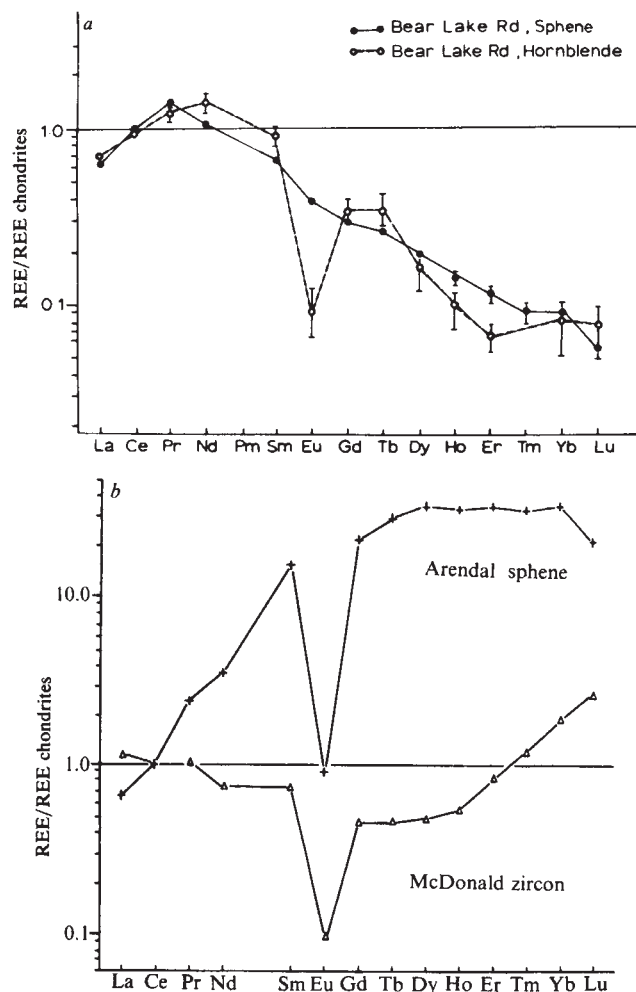


Fig. 2 *a*, Rare earth element distributions from a sphene, hornblende contact from Bear Lake Rd, Ontario, Canada. Intensities are divided by chondritic abundances from ref. 18 and normalized to cerium. In actual abundance $^{140}\text{Ce}^+$ sphene/ $^{140}\text{Ce}^+$ hornblende = 45. Error bars correspond to $\pm 1\sigma$. *b*, Rare earth element distributions from a sphene from Arendal, Norway and a zircon from the McDonald Mine, Ontario, Canada. Again intensities are compared to chondritic abundances and normalized to cerium.

the difference in energy distributions of the REEs and their oxides is not large compared with lighter elements, and the intensity loss with kinetic energy selection is again several orders of magnitude. Clearly, obtaining 'clean' elemental peaks with usable intensities is desirable, and this is the great advantage of the technique described below.

Our results were obtained on a Cameca IMS 3f instrument. A primary beam of $^{16}\text{O}^+$ was used exclusively at an accelerating voltage of 11.8–12.2 keV. The specimen isolation technique¹ requires the electrical isolation of the specimen (whether a conductor or insulator) from the sample holder, which in the case of a Cameca IMS 3f carries a secondary accelerating voltage of 4.5 keV. The specimen is thus allowed to charge under the primary beam, creating an extreme kinetic energy selection between the sample surface and the secondary accelerating field. This accounts for the high elemental ion/molecular ion discrimination observed. The lens effect of the sample holder is critical in maintaining usable secondary ion yields in these conditions¹. The beam conditions necessary to maintain an equilibrium charging rate impose some limitations on spatial resolution. Beam diameters of 40–50 μm in a spot mode, seem to yield optimum secondary ion intensity and stability. In all cases, lens settings in the primary column are determined solely from

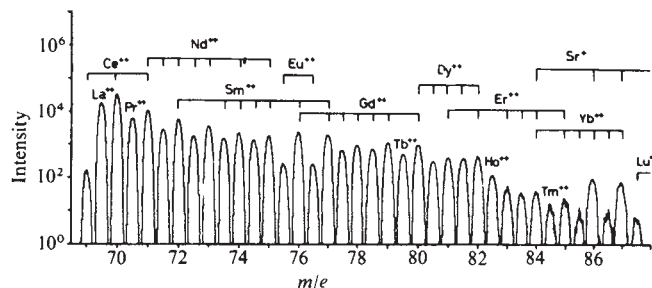


Fig. 3 The complete secondary ion mass spectrum of the monazite sample between $m/e = 69$ and 87, showing the doubly charged ions of the rare earth elements. Total spectrum, 1,000 channels, 5 s per channel.

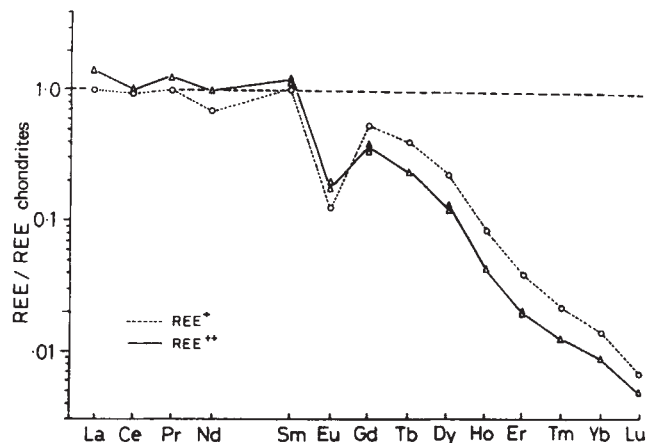


Fig. 4 The distribution of singly and doubly charged ions from the rare earth elements of the New Mexican monazite.

secondary ion yield and not by reference to the observed primary current. The apertures of the secondary column including the energy slits, remain fully open throughout analysis. This allows a considerable saving in intensity over the standard kinetic energy selection procedure⁸.

The six samples used in this study consisted of three sphene (CaTiSiO_5) samples, two from Ontario, Canada (Cardiff Mine and Bear Lake Rd) and one from Arendal, Norway, a hornblende in contact with one of the sphenes (from Bear Lake Rd), a zircon crystal from the McDonald Mine, Ontario and a monazite from New Mexico, USA. Count rates for the REEs ranged from 9.5×10^4 c.p.s. from Ce in the monazite to 4×10^2 c.p.s. for Ce in the Cardiff Mine sphene and 36 c.p.s. for Ce in Bear Lake hornblende. The baseline in all cases is < 0.1 c.p.s. typical of SIMS measurements.

This study initially concerned the mineral sphene because of its possible use as a host mineral for immobilizing radionuclides from nuclear fuel reprocessing wastes^{14,15}. The leaching behaviour of the REEs is considered to be a useful analogue for the behaviour of actinides in such structures. The study of REE distribution and fractionation is also of fundamental geological importance as described above.

Figure 1 shows a complete secondary ion mass spectrum of the Cardiff, Ontario sphene in which the REEs are clearly resolved. To confirm that the rare earth spectra are clear of molecular ions, isotope ratios can be derived for several rare earths (Table 1). The isotope ratios are in very good agreement with literature values¹⁶, particularly for the samples enriched in the heavy REEs (HREEs). There is little interference from hydrides and no measurable contamination of the HREE peaks from oxides of the light REE in the Arendal sphene, although $^{139}\text{La}^{16}\text{O}^+$ and $^{140}\text{La}^{16}\text{O}^+$ are probably present in the $^{155,156}\text{Gd}^+$ peaks of the Bear Lake sphene.

Despite the multitude of isotopes in the REE mass range and the resulting overlap at many mass units, each element in the

Table 2 Observed abundances for singly and doubly charged REEs from Monazite

Nd				Sm				Gd				Dy			
Mass	1+	2+	Natural abundance	Mass	1+	2+	Natural abundance	Mass	1+	2+	Natural abundance	Mass	1+	2+	Natural abundance
142*				144*			3.09	154*			2.15	160*			2.29
143	12.8	12.9	12.17	147	16.6	15.9	15.0	155	15.8	15.5	14.7	161	19.1	19.8	18.88
144*				148*			11.2	156	22.8	20.8	20.5	162	25.2	25.8	25.53
145	8.23	8.13	8.30	149	14.6	14.4	13.8	157	15.0	15.7	15.7	163	25.0	23.8	24.97
146	17.0	16.6	17.62	150*			7.44	158	22.1	23.6	24.9	164*			28.18
147*				152	24.3	25.1	26.7	160*			21.9				

* Peaks at these m/e values consist of an isotope from more than one element.

series has at least one 'clean' isotope. Assuming similar sputtering yields for the REEs, we can equate relative intensity to relative abundance and normalize to chondritic abundance in the usual way. Ion yields are not in fact equal for the REEs as Reed^{12,13} has shown. However, a discussion of ion yields in these conditions is beyond the scope of this paper. The resulting rare earth patterns are still a useful fingerprint of the sample and have semiquantitative significance.

The REE patterns (normalized to chondrites) of two sphenes, a hornblende and a zircon are illustrated in Fig. 2. The Bear Lake Rd. sphene and hornblende are of granitic origin and their patterns are similar to sphene and hornblende patterns obtained by others. For example, both the hornblende and zircon patterns are very similar to those observed by Gromet and Silver in hornblende and zircon samples from a Southern Californian granodiorite⁶. This gives the impression that each mineral displays a distinct REE pattern. However, comparison of the Bear Lake and Arendal sphene samples (Fig. 2a,b) demonstrates unequivocally that the REE patterns of minerals are determined as much by geological settings and processes as they are by mineral chemistry. This result is not surprising in that the geological history of the environment must influence both amounts and fractionation of constituents available to minerals during their formation.

The REE²⁺ spectra give strong confirming evidence that both REE⁺ and REE²⁺ peaks are indeed clean, and should provide important data for understanding ion yields and the fundamental processes of sputtering and ionization. We have observed the complete REE²⁺ spectrum for both the Arendal sphene and the monazite (Fig. 3). Peaks occur at integral and half mass units between $m/e = 69.5$ (¹³⁹La²⁺) and $m/e = 87.5$ (¹⁷⁵Lu²⁺). Isotope ratios of the 2⁺ ions are once again very close to natural abundances (Table 2), and are in very good agreement with the isotope ratios from 1⁺ ions. In both cases, there is some enhancement of the intensity of the lighter isotopes, as is observed for lighter singly charged ions. These data for the doubly charged ions are contrary to the observations of Shimizu and Hart¹⁷, who suggested heavy isotope enrichment for doubly charged ions from pure silver.

The REE²⁺ pattern when normalized to chondritic abundances (Fig. 4), falls below the REE⁺ pattern for the heavier rare earths, in line with the increasing second ionization potentials for the heavier rare earths.

Reed¹² has shown that ion yields for the REE⁺ ions do not simply follow ionization potentials, despite the chemical similarity of these elements. This affects particularly the light REEs La–Eu, and Lu which has both the lowest first ionization potential and the lowest yield. This pattern is clearly visible when comparing REEs with chondritic abundances, where deviations from a smooth curve (apart from Eu anomalies) are noted for La, Ce, Pr and Lu (Figs 2a,b and 4).

All our results suggest that rapid and reliable REE patterns of minerals can be obtained directly from SIMS results without evaluation of the absolute concentrations of these elements. Reed^{12,13} has shown variations in relative ion yields of the REEs but with suitable standards and/or better understanding of ion yields in specimen isolated conditions, quantitative analysis

could be achieved. The method is not competitive with neutron activation in sensitivity but for REE levels above 5–10 p.p.m. it offers significant advantages in speed, convenience and perhaps even in accuracy. The absence of background in the mass spectrum combined with improving stability of magnetic analysers mean count times and thus sensitivity can be increased significantly over the values reported here.

We thank Dr N. S. McIntyre for helpful discussion and D. Johnston and W. J. Chauvin of Surface Science Western for experimental assistance. This work was funded in part by Atomic Energy of Canada Ltd in support of the Canadian Nuclear Fuel Waste Management Program.

Received 2 August; accepted 5 October 1983.

- Metson, J. B., Bancroft, G. M., McIntyre, N. S. & Chauvin, W. J. *Surface Interface Analysis*, 5, 181–185 (1983).
- Frey, F. A., Bryan, W. B. & Thompson, G. *J. geophys. Res.* **79**, 5507–5527 (1974).
- Condie, K. C., Allen, P. & Narayana, B. L. *Contr. Miner. Petrol.* **81**, 157–167 (1982).
- Taylor, S. R. & McLennan, S. M. *Phil. Trans. R. Soc. A* **301**, 381–399 (1981).
- McLennan, S. M. & Taylor, S. R. *J. Geol.* **90**, 347–361 (1982).
- Gromet, L. P. & Silver, L. T. *Geochim. cosmochim. Acta* **47**, 925–940 (1983).
- Lovering, J. F. *Natn. Bur. Stand. Spec. Publ.* **427**, 135–178 (1975).
- Shimizu, N. & Hart, S. R. A. *Rev. Earth planet. Sci.* **10**, 483–526 (1982).
- Steele, I. M., Hervig, R. L., Hutcheon, I. D. & Smith, J. V. *Am. Miner.* **66**, 526–546 (1981).
- Shimizu, N., Semet, M. P. & Allégre, C. J. *Geochim. cosmochim. Acta* **42**, 1321–1334 (1978).
- Shimizu, N. *Nature* **289**, 575–577 (1981).
- Reed, S. J. B. *16th A. Meet. of the Microbeam Analysis Soc.*, Colorado (1980).
- Reed, S. J. B. *Int. J. Mass Spectrom. Ion Phys.* (in the press).
- Hayward, P. J. & Cecchetto, E. V. *Scientific Basis for Nuclear Waste Management* (ed. Topp, S. V.) (North Holland, Amsterdam, 1982).
- Nesbitt, H. W., Bancroft, G. M., Karkhanis, S. N. & Fyfe, W. S. *Scientific Basis for Nuclear Waste Management* Vol. 3 (ed. Moore, J. G.) 131–138 (Plenum, New York, 1981).
- Weast, R. G. (ed.) *Handbook of Chemistry and Physics* 58th edn (CRC Press, 1977).
- Shimizu, N. & Hart, S. R. *J. appl. Phys.* **53**, 1303–1311 (1982).
- Wedepohl, K. H. (ed.) *Handbook of Geochemistry* Vol II/5 (Springer, Berlin, 1970).

Evolution of the geomagnetic secular variation field from the beginning of the century

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Here we have monitored the secular variation (s.v.) of the geomagnetic field since the beginning of the century using observatory data. The energy of the s.v. field and the westward drift rate decreased from 1912–13 to 1969. Since 1969 these two parameters have again been increasing. The occurrence of jerks (steps in the second time derivative of the field) has been discovered at these two epochs (1912 and 1969). Moreover, after 1969, the s.v. field tends to have the same geographical distribution it had around 1912.

Several years ago it was shown that in 1969 the secular variation (s.v.) $\vec{B}(P, t)$ of the geomagnetic field $\vec{B}(P, t)$ (P is the current point at the Earth's surface, t is time) exhibited a sudden

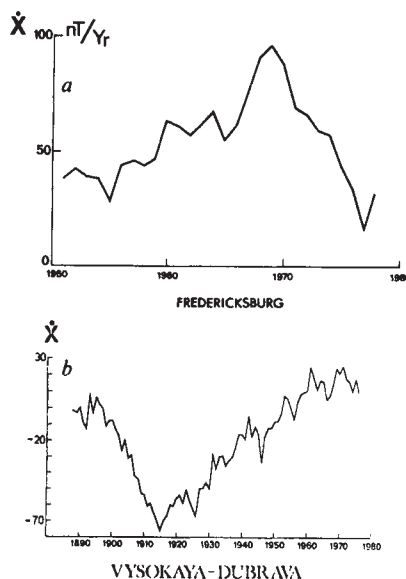


Fig. 1 *a*, The 1969 jerk as seen in the X component at the Fredericksburg observatory. *b*, The 1912 jerk as seen in the X component at the Vysokaya Dubrava observatory. The 1969 jerk at this location is less intense and not as clearly visible as in *a*.

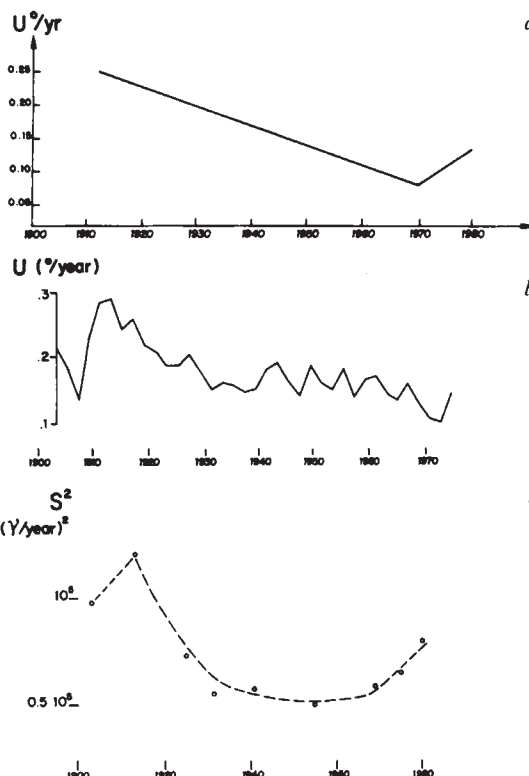


Fig. 3 *a*, Variation with time of the westward drift rate u computed from our simplified model of the geomagnetic secular variation. *b*, Variation with time of the westward drift rate computed using Hodder's method. *c*, Variation with time of the norm of the secular variation as given by spherical harmonic expansions.

change in slope—a step in the second time derivative of the field—of a clearly worldwide character¹⁻⁴. Figure 1*a* illustrates this event, called 'jerk' in English and 'secousse' in French.

More recently Ducruix *et al.*⁵ showed that a similar event occurred in 1912–13 (Fig. 1*b*). This event is, of course, less well documented than the 1969 one: only 50 observatories were operating at this time, among which only 10 were in the Southern Hemisphere. Nevertheless, Ducruix *et al.* were able to show that the 1912–13 jerk and the 1969 one have approximately the same geographical distribution, the signs of the second time derivative $\ddot{\mathbf{B}}(P, t)$ jump being reversed. Figure 2 illustrates these geographical distributions.

Ducruix *et al.*, from a close examination of all available secular variation curves, conclude that there is no other similar event over the 1912–80 time interval. Moreover, over this time span, the s.v. field appears to have a rather simple temporal variation: most of the corresponding curves are close to two straight lines, with a discontinuity in slope in 1969. Then, as a first approximation we may write:

$$\begin{aligned}\dot{\mathbf{B}}(P, t) &= \dot{\mathbf{B}}(P, 1912) + \dot{\mathbf{b}}(P) (t - 1912) & 1912 < t < 1969 \\ \dot{\mathbf{B}}(P, t) &= \dot{\mathbf{B}}(P, 1969) + \dot{\mathbf{b}}'(P) (t - 1969) & 1969 < t\end{aligned}\quad (1)$$

In many places, bumps of secular variation, with time constants typically of the order of some tens of years, are superimposed on the schematic s.v. field (1). They tend to obscure the simple broken line pattern which, nevertheless, represents the largest part of the observed s.v. field over the time span considered when studied over the whole Earth's surface.

Let us now choose a single global parameter characteristic of the s.v. field. It is natural to think first of the apparent westward drift (w.d.) rate u . We have computed, from 1912 to 1980, the w.d. rate corresponding to the simplified s.v. field (1) by minimizing quantities such as:

$$\delta^2 = \int \left\{ \left(\dot{\mathbf{B}}(P, t) - u(t) \frac{\partial \dot{\mathbf{B}}}{\partial \phi}(P, t) \right)^2 \right\} dS \quad (2)$$

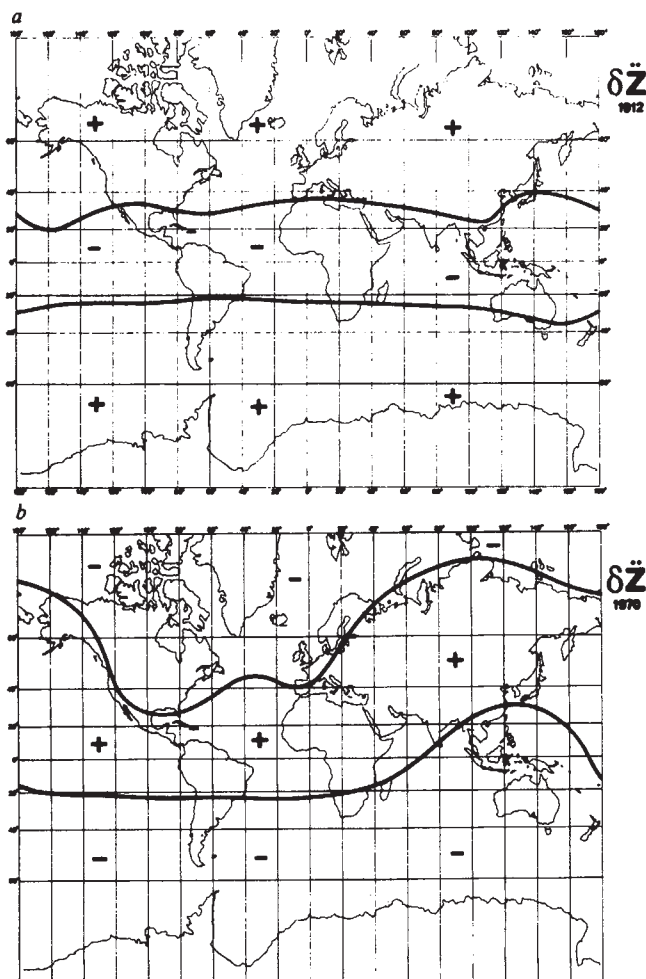


Fig. 2 Comparison between the geographic distributions of the jerks of 1912 and of 1969. The quantity $\delta \ddot{Z}$ is the amplitude of the discontinuity of the second time derivative. Only the zero isopleths are shown.

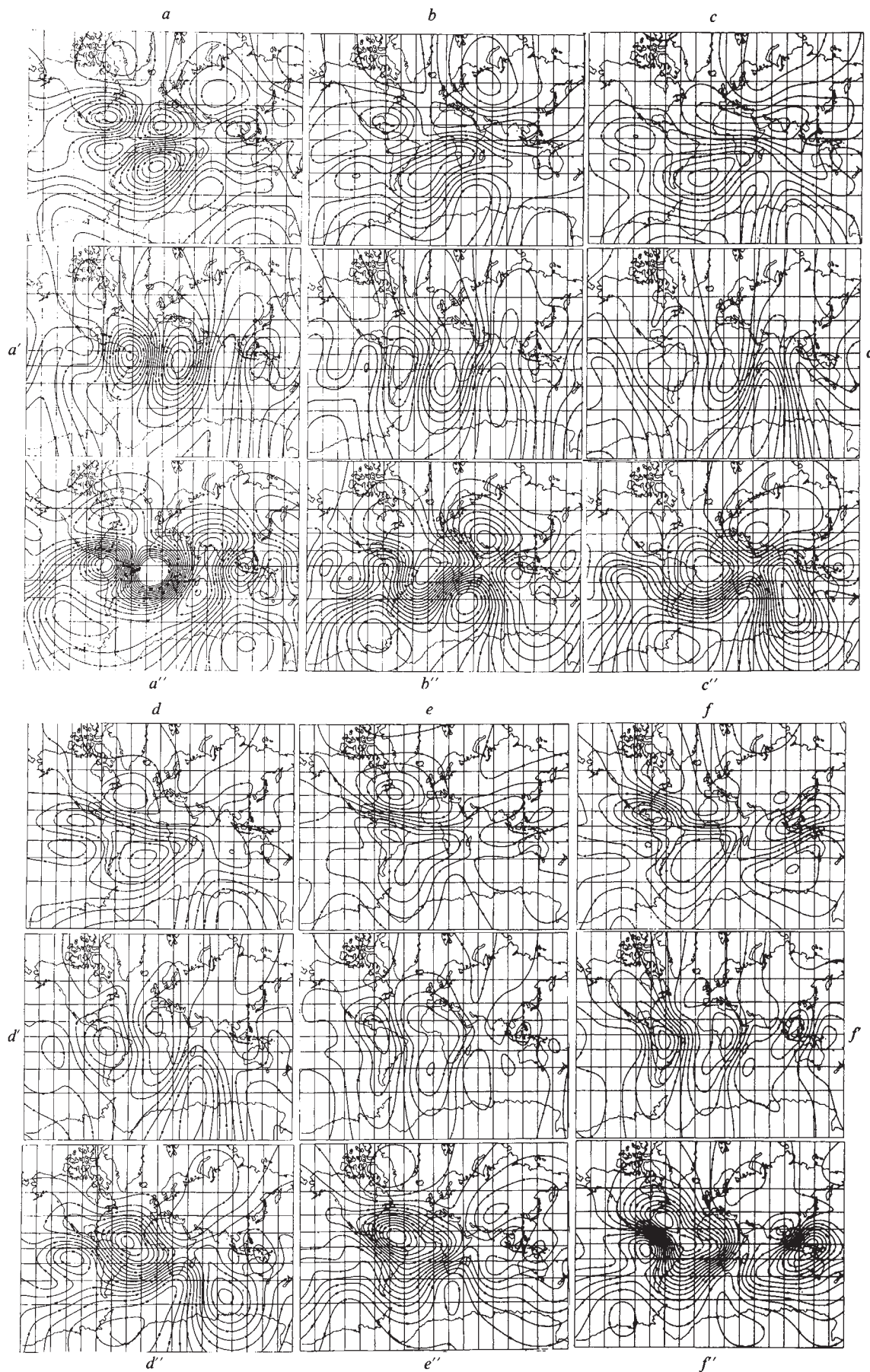


Fig. 4 Evolution with time of the maps of the secular variation for epochs 1913, 1925, 1941, 1955, 1969, 1980 as computed by Hodder. The first row displays the secular variation of X ($a-f$), the second row s.v. of Y ($a'-f'$), the third row s.v. of Z ($a''-f''$). The contour interval is 20 nT (yr)^{-1} .

The results are illustrated in Fig. 3a. It has previously been shown that the problem of determining u by minimizing quantities such as equation (2) is an ill-posed problem and gives somewhat different values when different techniques and different components of $\vec{B}$ are used. However, we are only interested here in the trend of the global parameter u from 1912 to 1980—a decrease from 1912 to 1969, then an increase. The 1912–69 decrease also appears in Hodder's estimates⁸ derived from raw observatory data (Fig. 3b).

Another global parameter is simply the norm $S^2 = \sum_{n,m} (\dot{g}_n^{m^2} + \dot{h}_n^{m^2})(n+1)$ of the s.v. field $\vec{B}$. [$(\dot{g}_n^m, \dot{h}_n^m)$ are the coefficients of the spherical harmonic expansion of $\vec{B}$ with Schmidt's normalization]. Figure 3c represents the variation of S^2 with time using s.v. coefficients given by Hodder⁹ which rely on observatory data, except in 1980 where the GSFC 1980 s.v. coefficients are used. It appears that the energy decreases steadily from 1912 to 1945, is then nearly constant from 1945 to 1969 (as shown by Fig. 1 of Hodder's paper⁹, the lack of homogeneity of the different sets of coefficients can to some extent bias the curve $S^2(t)$, but the general trend can be trusted) and finally increases again after 1969. This confirms the importance and worldwide character of the 1912–13 and 1969 events.

Let us now look at the values of the s.v. field as illustrated by isovalue lines of components $\dot{X}$ (north), $\dot{Y}$ (east) and $\dot{Z}$

(vertical) of $\vec{B}$ for different epochs from 1912 to 1980. The maps in Fig. 4 have been computed, again using s.v. coefficients given by Hodder as above (except for 1980). Despite the uncertainty in the contours of these charts, it is clear that, from the 1912 high of u (or of S^2) to the 1969 low (Fig. 3a, b), the shape of the s.v. field is evolving monotonically; in particular, the isovalue lines get looser and looser, and the s.v. field becomes 'exhausted'. Then the 1969 jerk occurs and the s.v. field increases again and tends to recover the strength it had in 1912. Furthermore, the s.v. field tends to recover, after 1969, the value it had around 1913, at the time of the high of u : the broad lines of maps a, a', a'' and maps f, f', f'' of Fig. 4 indeed present striking similarities (the three components must be considered together) except over the Far-East. Part of the discrepancies could be due to the poor control of 1913 contour lines over these regions.

Unfortunately, these observations are limited to a short time interval. It is, of course, desirable to go back further into the past, but field analysis then relies increasingly heavily on European observatories (19 observatories were operating in 1900, 12 of which were in European countries).

Theoretical studies of the hydrodynamics of the outer core should now account for the occurrence of the 1912–13 and 1969 jerks and for the dramatic change of the secular variation from 1969 to 1980.

5. Ducruix, J., Gire, C. & le Mouél, J. L. *C.r. hebdomadaire. Séances Acad. Sci., Paris* **296** Ser. II, 1419–1424 (1983).

6. le Mouél, J. L. & Courtillot, V. *J. Geophys. Res.* **87**, 4103–4108 (1982).

7. Gire, C., le Mouél, J. L. & Madden, T. A. *Geophys.* (submitted).

8. Hodder, B. M. *Nature* **306**, 136–137 (1983).

9. Hodder, B. M. *Geophys. J. R. astr. Soc.* **65**, 763–776 (1981).

Received 19 September; accepted 6 October 1983.

1. Courtillot, V., Ducruix, J. & le Mouél, J. L. *C.r. hebdomadaire. Séances Acad. Sci., Paris* **D287**, 1095–1098 (1978); **B289**, 173–175 (1979).

2. Ducruix, J., Courtillot, V. & le Mouél, J. L. *Geophys. J. R. astr. Soc.* **61**, 73–94 (1980).

3. Malin, S. R. C. & Hodder, B. M. *Nature* **296**, 726–728 (1982).

4. Backus, G. E. *Geophys. J. R. astr. Soc.* **74**, 713–746 (1983).

Oldest reliable $^{40}\text{Ar}/^{39}\text{Ar}$ ages for terrestrial rocks: Barberton Mountain komatiites

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We report here the first $^{40}\text{Ar}/^{39}\text{Ar}$ ages for komatiites and komatiitic basalts from the Barberton Mountain Greenstone Belt on the Transvaal–Swaziland border in southern Africa¹. Both rock types display remarkable argon retentivity. While some variation is found among the samples, the best argon age estimate for the time of metamorphism is in the 3,450–3,490 Myr range. This age is only slightly less than that found in komatiites from the same area by Sm–Nd dating (3,540 ± 15 Myr)². Within the uncertainties of the decay constants involved, the two age estimates are not different. The results show that the principal pervasive greenschist metamorphism¹ in the area must have occurred within 100 Myr of the eruption of the komatiite. Analytically precise ages were obtained despite potassium contents as low as 80 p.p.m. in the presence of high calcium concentrations (3–5% Ca). To our knowledge, the results represent by far the oldest reliable ages obtained for terrestrial rocks using the K–Ar system.

Komatiites have attracted widespread interest among earth scientists³ because of their unusual geochemistry and mineralogy, and because they are predominantly confined to the Archaean. The Barberton Greenstone Belt contains, in the Onverwacht Group at the base of the Swaziland Supergroup, one of the earliest and best preserved komatiitic sequences. While the time of eruption of these lavas is usually considered to have been established by Sm–Nd dating² (Rb–Sr⁴ and U–Pb⁵ dates were much less precise), the subsequent tectonothermal

Table 1 Plateau and integrated ages of komatiite samples

Sample	Integrated age (Myr)	Plateau age (Myr)	% ^{39}Ar release*
Komatiites			
B40A	3,367 ± 8	3,463 ± 7	72
B40A	3,336 ± 4	3,486 ± 3	59
B40A	3,368 ± 15	3,425 ± 17	54
B40A	3,346 ± 11	3,429 ± 10	63
B40A	2,961 ± 14	3,327 ± 9	75
B40A	3,539 ± 55	3,406 ± 37	92
B41B	3,312 ± 25	3,407 ± 17	63
B40E	2,986 ± 6	3,318 ± 7	44
B40E	3,060 ± 5	3,420 ± 6	33
KT7G	2,905 ± 8	3,225 ± 8	29
KT7G	2,962 ± 7	3,298 ± 10	30
KT16A	2,816 ± 3		
KT16A	2,814 ± 2		
Komatiitic basalts			
KT17B	3,778 ± 2	3,428 ± 2	64
KT17B	3,777 ± 2	3,447 ± 2	54
KT17A	3,053 ± 3	3,111 ± 3	53

Decay constants from ref. 14 were used. Errors are ±1σ.

* Per cent of ^{39}Ar release involved in plateau age calculation.

history has been uncertain. To shed light on the situation, we have carried out a detailed $^{40}\text{Ar}/^{39}\text{Ar}$ age investigation on ultramafic and mafic lavas from this area.

We made 13 step-heating analyses of five different komatiite specimens, and three similar analyses of two different komatiitic basalts, all from the type area¹ of the Komati Formation of the Onverwacht Group. Whole-rock samples, of ~250 mg mass, were irradiated for ~150 h in the McMaster University research reactor. Samples of hornblende 3 gr⁶ were irradiated as standards alongside the unknowns and were fused with the Toronto Laser Probe⁷ in one-step runs. Irradiated komatiites and komatiitic basalts were step-heated to 1,200 °C using the detailed temperature control vacuum system described by Berger and York⁸. Residues were then fused in a radiofrequency

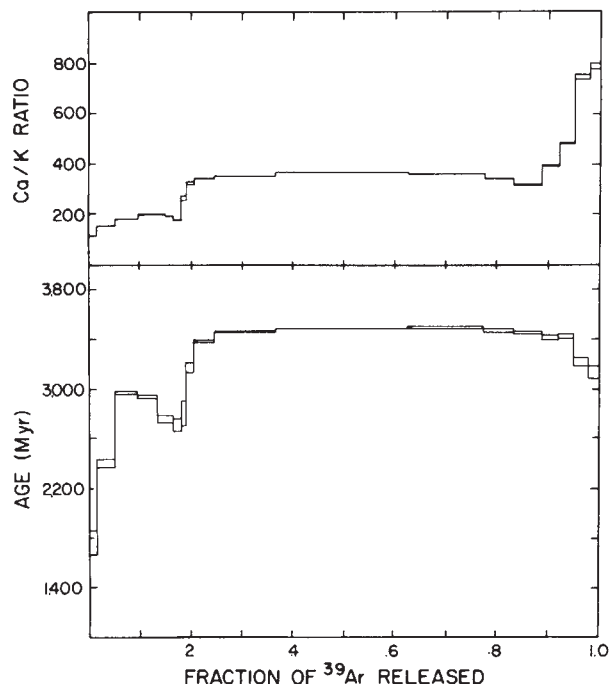


Fig. 1 Age and Ca/K spectra of vacuum-stored komatiite sample B40A. The sizes of the boxes represent 1σ errors, where they are large enough to be shown.

system. Argon isotope ratios in the purified gases were measured with an MS10 mass spectrometer. During the initial analyses it was discovered⁹ that the komatiite samples tended to absorb modern atmospheric argon in large quantities after irradiation. A dramatic reduction of this effect was achieved in the later runs by storing the komatiites under vacuum after irradiation until they were required for analysis. These later runs were therefore of higher precision.

The plateau and integrated ages (integrated age = age calculated from the ratio of the total radiogenic ^{40}Ar in the sample to the total potassium-derived ^{39}Ar) are presented in Table 1. Figure 1 shows a typical age spectrum for the komatiites, and the results of duplicate analyses of a komatiitic basalt are shown in Fig. 2. Detailed analytical data will be presented elsewhere.

From Table 1 it can be seen that the komatiites have lost some argon, as the integrated ages are (with one exception out of 13) significantly less than the Sm-Nd age of $3,540 \pm 15$ Myr. Despite this, plateau ages approaching 3,500 Myr are obtained by the step-heating approach.

The first sample examined, B40A, has been studied in the greatest detail. The fifth and sixth runs on this sample in Table 1 were our earliest⁹ and are not of as high a quality as the top four runs listed, which were performed after techniques appropriate to komatiites had been devised. These four superior analyses all yielded integrated ages between 3,300 and 3,400 Myr. It is interesting that two of these four samples, having been vacuum-stored after irradiation, suffered accordingly much lower modern atmospheric argon contamination and yielded the highest plateau ages: $3,463 \pm 7$ and $3,486 \pm 3$ Myr. The latter age corresponds to the plateau in the age spectrum illustrated in Fig. 1. In this analysis we extracted gas in several small steps at both ends of the obvious age plateau in order to minimize mixing of adjacent off-plateau gas of low age with the true plateau. Therefore, it may be significant that this extremely precise plateau age (the error in the plateau age = 3 Myr (1σ)) is also the oldest we have yet found.

The shape of the age spectrum in Fig. 1 is characteristic of all those found in the komatiite specimens, except for KT16A, which will be referred to below. This characteristic age spectrum contains three totally distinct portions, with three correspondingly distinct segments in the Ca-K spectrum (shown in the top part of Fig. 1). The low temperature release (600–800 °C)

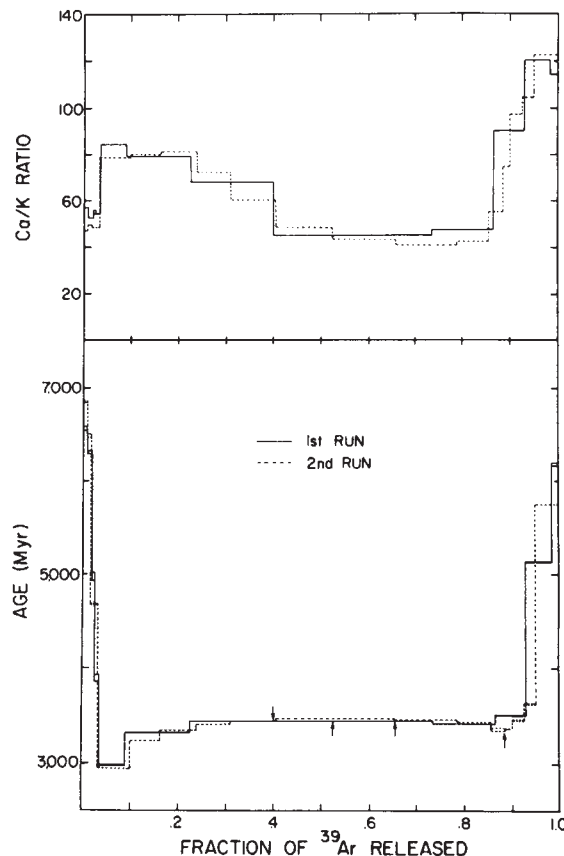


Fig. 2 Age and Ca/K spectra of vacuum-stored komatiitic basalt sample KT17B. Where they are large enough to be shown, the 1σ errors are represented by the sizes of the boxes. Arrows indicate divisions between two fractions of equal age. Note that the Ca/K values and the shape of the Ca/K spectrum are very different from those found in the komatiite samples (see Fig. 1).

displays relatively low ages and Ca/K ratios of ~ 200 . At intermediate temperatures (925–1,035 °C) the typical age plateau appears, together with a new Ca/K regime having a value in the range 350–500 depending on the sample. At the highest temperatures ($\geq 1,100$ °C) there is a slight but experimentally reproducible dip in the age along with a large increase in Ca/K ratios. Petrographically, the komatiites retain some primary textures, but consist almost entirely of metamorphic assemblages: serpentine-tremolite-chlorite-magnetite and very minor ferristilpnomelane. Electron microprobe X-ray scans and quantitative analyses show that the only mineral present in sufficient quantity in the komatiite, with the required Ca/K ratio to account for the plateau segment in the age spectrum, is tremolite. The younger ages in the low temperature release range are correlated with the presence of stilpnomelane.

Komatiite specimen B41B also has an integrated age of over 3,300 Myr and displays a plateau age of $3,407 \pm 17$ Myr, the slightly larger error here being due to the fact that this sample has the lowest potassium content (80 p.p.m.) of all the komatiites examined.

The remaining three komatiites (B40E, KT7G and KT16A) all exhibit greater argon losses from their whole-rock systems, having integrated ages in the 3,060–2,814 Myr range. The lowest such value (2,814 Myr) was obtained for KT16A which, presumably because of its severe disturbance, also displays no plateau in its age spectrum. In contrast, samples B40E and KT7G do show age plateaus, in the 3,420–3,225 Myr range, despite large total argon losses.

The two komatiitic basalts analysed behave completely differently from the komatiite samples and from each other. KT17A shows severe argon loss (integrated age = $3,053 \pm 3$ Myr) and a ragged age spectrum with a crude plateau at 3,100 Myr. In complete contrast with this, and with all the komatiites so far

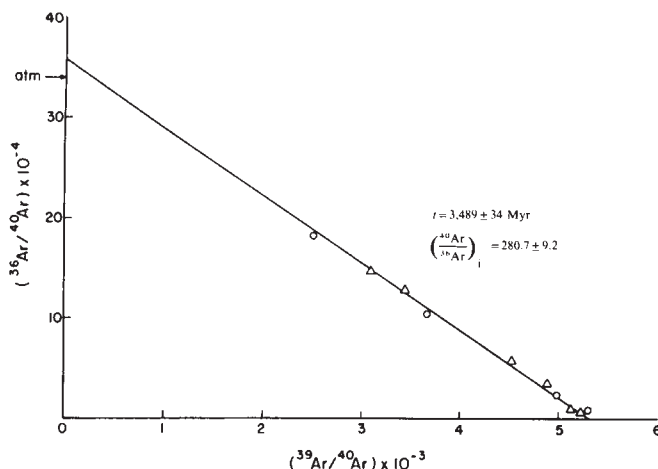


Fig. 3 Isotope correlation plot for the data from the plateaux of the high-precision vacuum-stored analyses of B40A. Except for two points where the errors are approximately the same size as the symbols, the errors in the data are less than the dimensions of the symbols. ○, First run; △, second run.

analysed, komatiitic basalt KT17B contains excess radiogenic argon. Thus, duplicate step-heating analyses gave integrated ages of $3,777 \pm 2$ and $3,778 \pm 2$ Myr, ~ 240 Myr older than the Sm–Nd age. However, as may be seen in Fig. 2, the step-heating experiments are remarkably successful in removing the effect of the excess argon. The well known saddle-shaped spectrum frequently found in samples containing excess argon¹⁰ is observed. A very broad well-defined plateau is revealed in the middle temperature (900–975 °C) fractions, yielding plateau ages of $3,428 \pm 2$ and $3,447 \pm 2$ Myr for the duplicates. The latter value was obtained in an experiment in which we took extra, small steps at both ends of the age plateau to reduce the effect of mixing off-plateau gas with the plateau fractions. As with B40A (referred to above) this did seem to produce a slightly higher plateau age.

The komatiitic basalts are comprised of actinolite with rims and patchy intergrowths, crystallographically controlled, of ferro-hornblende, epidote-choked albite, chlorite and altered titanomagnetite with associated minor ferristilpnomelane. Electron microprobe analyses, coupled with the Ca/K spectra from the age runs, show that the age plateaux are dominated by argon release from hornblende, while disturbed portions of the spectra appear related to stilpnomelane. As far as we know, this is the first report of stilpnomelane in either Barberton komatiites or komatiitic basalts, and indicates a condition of low oxygen fugacity during the main metamorphism, with the stilpnomelane having originally formed as ferrostilpnomelane¹¹. The later oxidation to ferristilpnomelane may be reflected in the younger ages in the low temperature release range of the komatiites.

With the exception of komatiite sample KT16A which retained some primary olivine, all the samples consist of metamorphic mineral assemblages. We conclude, therefore, that the tremolite in the komatiites and the hornblende in the komatiitic basalt (KT17B) both record the time of the metamorphism of the Komati Formation. From the superior analyses of B40A and KT17B we place this metamorphism somewhere in the 3,450–3,490 Myr age range (within experimental errors). Evidently this principal, pervasive metamorphism¹ occurred within less than 100 Myr of eruption (possibly almost immediately after, when uncertainties in the ^{40}K and ^{147}Sm half lives are considered) of the komatiites. Chauvel *et al.*¹² have recently suggested that in certain circumstances Sm–Nd dates on komatiitic rocks may be anomalously old. Our data indicate that this is not the case here.

All our measurements were corrected for instrumental argon blanks. Any ^{36}Ar remaining after that was used to make a modern atmospheric argon correction. *A priori*, it was, of course, not obvious to us that this would be the correct procedure.

Conceivably, the samples may have contained initial argon having a $^{40}\text{Ar}/^{36}\text{Ar}$ ratio totally different from that found in today's atmosphere. That no significant error has been introduced, however, is apparent from the consistency of the data in the presence of a wide range of ^{36}Ar -based corrections. To test this point further and indeed to look for primordial argon, we examined the distribution of the data from the plateaux of the high-precision vacuum-stored B40A runs (the top two analyses in Table 1) on a graph of $^{36}\text{Ar}/^{40}\text{Ar}$ versus $^{39}\text{Ar}/^{40}\text{Ar}$ (Fig. 3). The best straight line¹³ allowing for all reasonable sources of correlation in the errors, gives an age of $3,489 \pm 34$ Myr. While the data are highly linearly correlated, they are more scattered about the best line than would be expected on the basis of experimental error alone. The intercept on the $^{36}\text{Ar}/^{40}\text{Ar}$ axis indicates initial argon in the samples with a $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of 280.7 ± 9.2 (1σ , where the error allows for the scatter of the samples about the line). While this is lower than the modern atmospheric value of 295.5, the difference is not statistically significant. Only more detailed studies will show whether this intercept indicates some sort of primordial argon or whether it represents a mixture of initial argon heavily contaminated with modern atmospheric argon, despite our attempt to eliminate this by post-irradiation vacuum storage. Until such measurements have been performed, the data do not allow us to favour any particular one of the theories of mantle–atmosphere evolution.

Finally, as mafic and ultramafic rocks (both komatiitic and tholeiitic) constitute volumetrically the bulk of Archaean greenstone belts, our demonstration here of the precise dating of the greenschist metamorphism of individual komatiitic lavas indicates the potential value of the $^{40}\text{Ar}/^{39}\text{Ar}$ dating method in elucidating the tectonothermal history of these terrains.

We thank Maarten J. de Wit and Chris J. Hale for supplying the samples, and W. John Kenyon and Peter Kuybida for technical assistance. This research was funded by grants to D.Y. and J.A.H. from the Natural Science and Engineering Research Council of Canada, and by a grant to J.A.H. from the Queen's University Advisory Research Committee. M.L.M. was supported by the Consejo Nacional de Ciencia y Tecnologia (Mexico).

Received 28 July; accepted 26 October 1983.

1. Viljoen, M. J. & Viljoen, R. P. *Geol. Soc. S. Afr. spec. Publ.* **2**, 9–28, 55–85 (1969).
2. Hamilton, P. J., Evensen, N. M., O'Nions, R. K., Smith, H. S. & Erlank, A. J. *Nature* **279**, 298–300 (1979).
3. Arndt, N. T. & Nisbet, E. G. *Komatiites*, 526 (Allen & Unwin, London, 1982).
4. Jahn, B. M. & Shih, C. Y. *Geochim. cosmochim. Acta* **38**, 873–885 (1974).
5. Van Niekerk, C. B. & Burger, A. J. *Trans. geol. Soc. S. Afr.* **72**, 9–25 (1969).
6. Hall, C. M., Walter, R. C., Westgate, J. A. & York, D. *Nature* (in the press).
7. York, D., Hall, C. M., Yanase, Y., Hanes, J. A. & Kenyon, W. J. *Geophys. Res. Lett.* **8**, 1136–1138 (1981).
8. Berger, G. W. & York, D. *Geochim. cosmochim. Acta* **45**, 795–811 (1981).
9. York, D., Hall, C. M., Masliwec, A., Langridge, R. L. & Hale, C. J. *EOS* **62**, 429 (1981).
10. Lanphere, M. A. & Dalrymple, G. B. *Earth planet. Sci. Lett.* **32**, 141–148 (1976).
11. Brown, E. H. *Contr. Miner. Petrol.* **31**, 275–299 (1971).
12. Chauvel, C., Dupre, B., Todt, W., Arndt, N. T. & Hofmann, A. W. *EOS* **64**, 330 (1983).
13. York, D. *Earth planet. Sci. Lett.* **5**, 320–324 (1969).
14. Steiger, R. H. & Jäger, E. *Earth planet. Sci. Lett.* **36**, 359–362 (1977).

Dating diagenesis in a petroleum basin, a new fluid inclusion method

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The final porosity and permeability of sandstone petroleum reservoirs is greatly affected by the diagenetic growth of minerals after deposition. For example a sand may be deposited with a porosity of 25% and a permeability of 5,000 mdarcy (mD)¹; diagenetic growth of quartz around detrital sand grains may leave a rock with only 10% porosity, and later growth of

clays may partly fill these remaining pores and block inter-pore connections, reducing permeability to 100 mD (ref. 2). If the depth and timing of such diagenetic alteration can be measured and the extent of diagenesis estimated, then prediction of the diagenetic state of undrilled sandstones may become possible and diagenesis related more closely to the timing of hydrocarbon migration and the formation of hydrocarbon traps. We present an example of a new method for estimating the date of quartz diagenesis using a combination of techniques from thin section petrography, fluid inclusion thermometry, organic geochemical thermometry and sedimentary basin stratigraphic analysis. These results suggest that quartz in the Beatrice oilfield was precipitated from moving and cooling pore fluids, at a temperature between 68 °C and 94 °C in the late Jurassic.

The Beatrice oilfield, operated by Britoil, lies in the Inner Moray Firth of the UK North Sea and contains oil reservoirs in middle Jurassic sandstones³. Thin section and scanning electron microscope (SEM) studies of the diagenetic sequence in Bajocian reservoir sandstones indicate a specific diagenetic sequence: early-formed concretionary calcite, minor feldspar leaching and kaolinite formation, syntaxial quartz overgrowth (in at least two phases), widespread feldspar leaching and coeval kaolinite formation with minor illitization of some muscovite, minor late aggressive calcite and finally minor very late cryptocrystalline silica. The details of diagenesis will be described in more detail elsewhere⁴, but the general sequence is typical of many sandstones both in the North Sea, and in other petroleum basins^{5,6}.

Thin section observations show that the primary permeability in some sandstone layers has been low (1–3 mD) since deposition, due to blockage of pore connections by detrital clay. These sandstones show very little diagenetic alteration and contain 3–4% potassium feldspar. By contrast, compositionally similar sandstones with high (>100 mD) primary permeabilities have experienced dissolution of potassium feldspar, and growth of authigenic quartz to form about 13%, by area, of the whole rock. Only 1% of grains in all these sandstones have suffered any pressure solution. Quartz overgrowth has uniformly affected the permeable sandstones, except in areas immediately beneath thin (5–50 cm), laterally persistent, mudstone beds, where diagenetically overgrown quartz forms a pervasive 3–20 cm thick layer, so that quartz forms about 99% of the rock. Similar layers do not occur above these mudstones. Such features, where diagenesis is limited to permeable sandstones and enhanced below impermeable layers, suggest that the diagenetic pore fluids moved through the sandstones and precipitated quartz as they cooled beneath the mudstones. It may be noted that in neutral to acid fluids, the solubility of quartz at diagenetic temperatures and pressures is dominated by temperature⁷, rather than being dependent on ionic concentration^{6,7}, salinity⁸, pressure⁷, or pH changes⁷.

During the formation of diagenetic quartz overgrowths, samples of pore fluids were trapped as fluid inclusions and as they show no signs of leakage, each is assumed to have been constant in volume and composition since its formation. These 'primary' inclusions were examined by heating and freezing fluid inclusion microscopy^{9,10}. Briefly, in the case of a simple low salinity aqueous fluid, this method involves cooling an inclusion to freeze its fluid, and then warming it. The salinity of the fluid^{10,11} is given by the temperature at which the ice crystals melt, and other chlorides present can be expressed as equivalent NaCl¹⁰. Further heating causes the inclusion to revert to a one-phase system at the 'homogenization temperature', which is the minimum trapping temperature of the inclusion and hence the fluid density¹⁰ is determined. The true trapping temperature of an inclusion was hotter and lay somewhere along the constant density 'isochore' path through the liquid or fluid phase for the inclusion concerned. This line can be drawn from pressure-volume-temperature properties of the NaCl–H₂O fluid^{10,15,16} and its measured density. In this paper the size of the upward temperature correction is constrained using an independent organic geochemical measurement of the maximum temperature

Table 1 Polycyclic alkane data used to establish maximum palaeotemperatures for the interbedded mudrocks

Sterane and triterpane ratios	Ratio* (%)	Equivalent† temperature (°C)	Comments on plots used‡
Ratio Tt No. 5	22.4	78	Well defined linear low scatter plot (Fig. 2).
Ratio St No. 5	40.8	94	Linear portion of moderate scatter plot
Ratio St No. 6	50.1	97	On shoulder of moderate scatter plot
Ratio St No. 9	38.6	87	Well defined sigmoidal low scatter plot

The following ratios have been measured for the interbedded mudstones. Triterpanes: Ratio Tt 5: 17 β (H), 21 α (H), 30-normoretane + [17 β (H), 21 α (H), 30-normoretane] + [17 α (H), 21 β (H), 30-norhopane]. Steranes: Ratio St 5: 5 α , 14 α , 17 α methyl cholestanes (20R + 20R + 20S). Ratio St 6: 5 α , 14 α , 17 α ethyl cholestanes (20R + 20R + 20S). Ratio St 9: 5 α , 14 β , 17 β , 20R + 20S ethyl cholestanes + (5 α , 14 β , 17 β , 20R + 20S) + (5 α , 14 α , 17 α , 20R + 20S) ethyl cholestanes.

* Quantitations based on *m/e* 191, 386, 217 and 218 (ref. 12, Table 1).

† Error on measurements ± 6.5 °C (see text).

‡ Taken from calibration plots in ref. 12.

experienced by interbedded mudstones. This enables a pressure measurement at the time of trapping to be derived, rather than the usual stratigraphical method being used, of estimating a pressure to give a temperature. The maximum temperature recorded by the organic geothermometer may have been reached later than the time of trapping; however, since the organic temperature is only used as a constraint on the upward limit of the homogenization-to-trapping temperature correction, this will tend to give maximum trapping temperatures and maximum pressures.

In the Beatrice Field, inclusion salinities range from 8 to 17 equivalent weight per cent NaCl¹¹ and homogenization temperatures show a unimodal distribution around 60–78 °C, $\bar{x}$ = 68 °C (Fig. 1), and show no variation with position in the overgrowths or between different parts of the reservoir sandstones. In this case organic rich mudrocks are interbedded with the reservoir sandstones, so that the maximum burial temperature can be estimated from changes in the molecular ratios of triterpanes and steranes^{12,13}. Four organic geothermometer polycyclic alkane ratios have been measured for the interbedded mudstones (Table 1). These ratios have been compared with trends established from a depth (temperature) sequence of Kimmeridge Clay Formation (North Sea 'hot shales') to determine the maximum temperature of burial for the interbedded mudstones. Although the depositional environment, and to some extent the kerogen type of the middle Jurassic interbedded mudstones in Beatrice are not identical to the Kimmeridge Clay Formation, both contain kerogens of an algal origin, bacterially degraded to a lesser or greater extent.

It should be noted that the polycyclic alkane ratios used are those that give a relatively low level of scatter in the calibration plots (Table 1, Fig. 2), and are sensitive to temperature in the range under consideration. Within our laboratories a duplicate analysis on a split sample of powdered sediment generally gives a reproducibility of $\pm 5\%$ on the ratios used, which corresponds to an average difference in temperature of ± 6.5 °C over the temperature range under discussion. The use of the calibration curves to define the maximum palaeotemperature is entirely pragmatic, and does not rely on, or imply, any precursor-product relationship between the ratioed molecules (contrast ref. 14). Use of the Kimmeridge Clay Formation calibration plots for the middle Jurassic shales is justified by the good agreement

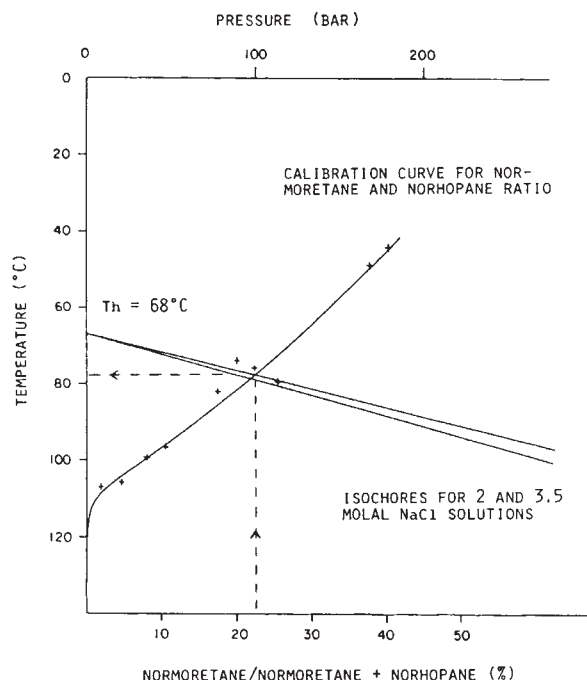


Fig. 1 Homogenization temperatures of fluid inclusions trapped in diagenetic quartz overgrowths from middle Jurassic oil reservoir sands of the Beatrice field, North Sea. Inclusions at different positions within overgrowths give similar homogenization temperatures. The spread of values may be due to slight 'necking' of some inclusions. Blank = aqueous inclusions; black = hydrocarbon bearing inclusion. Number of inclusions = 38, $\bar{x}$ (total) = 67.6 °C, $\bar{x}$ (central peak, $55 \leq Th \leq 80$) = 67.1 °C, $\sigma = 5$ °C.

between the predicted temperatures for the four essentially independent ratios. Use of these four ratios indicates a minimum temperature of 78 °C and a maximum of 97 °C with a mean of 89 °C. However, the lower values derive from the calibration curves showing the least degree of scatter and are hence in practice likely to be more accurate.

We are now in a position to constrain the maximum trapping temperature at which fluid inclusions formed, and to derive the pressure at which they formed. The polycyclic alkane ratio with the best calibration curve gives a maximum temperature of 78 °C (Table 1). Thus the most probable correction of fluid inclusion homogenization temperature is from 68 °C to 78 °C, i.e. 10 °C. As the density of the trapped fluid can be calculated^{15,16}, and the volume of an inclusion has remained constant from its time of formation, this correction to a maximum temperature of 78 °C equates to a maximum burial pressure of 100 bars (Fig. 2)^{15,16} which is equivalent to a depth of 1.0 km under purely hydrostatic loading, or of 0.4 km under purely lithostatic loading (mudrock density 2.4 g cm⁻³). However, pressures at 0.4 km are unlikely to be lithostatic¹⁷ and the necessary geothermal gradient of about 195 °C km⁻¹ is very unlikely. Thus we are forced to conclude that diagenetic quartz overgrowth occurred at about 1.0 km depth under hydrostatic pressure, which enabled free circulation of diagenetic fluids. If the less reliable temperature of 94 °C is used (Table 1) as a maximum constraining estimate, the maximum temperature correction becomes 26 °C and so maximum pressure of quartz overgrowth increases to 240 bar, equivalent to between 1.0 km (lithostatic) and 2.4 km (hydrostatic). However, we suggest that since hydrostatic pressures are unlikely below 1.5 km (ref. 17), quartz overgrowth formation did not occur below this depth.

The shape¹⁸ and stratigraphy³ of the basin are known from seismic and drilling evidence. Subsidence in the Beatrice area was slow during deposition of the reservoir sandstones and remained slow until the earliest Oxfordian¹⁹, accumulating 180 m of sediment (present-day thicknesses). Subsidence rates increased dramatically in the Oxfordian, culminating in late Oxfordian sedimentation rates of about 100 m per Myr, and

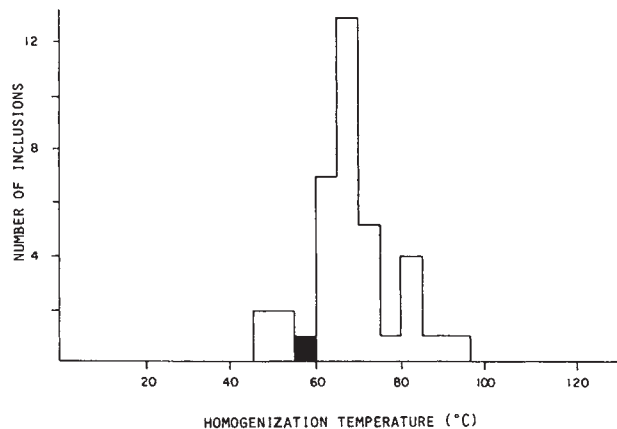


Fig. 2 Limits on pressure and temperature of diagenetic quartz overgrowth formation from volumetric properties¹⁰ of system NaCl-H₂O. Mean inclusion homogenization temperature of 68 °C shown, together with dashed line for maximum sediment temperature of 78 °C derived from ratio Tt 5 (Table 1), calibrated using North Sea Kimmeridge Clay Formation (+) isomer data¹². Note that triterpane ratios are calibrated against temperature rather than pressure, the correspondence of values on the pressure and triterpane scales being expected to vary for different sedimentary basins.

accumulating 340 m of Oxfordian mudstones in all. Subsidence slowed during the Kimmeridgian¹⁹, accumulating 200 m of mudrock, but became rapid again during the Tithonian¹⁹, preserving 320 m of mudrock. During this late Jurassic basin subsidence a total of 860 m of mudrock accumulated in about 19 Myr (ref. 19).

We considered two extreme cases, maximum and minimum organic geothermometric temperatures, with overburden pressures derived from fluid inclusions, and equated these with overburden burial pressures generated by the present-day sediment thicknesses. Case 1: an organic geothermometric temperature of 78 °C implies, from fluid inclusion data, an overburden pressure of 100 bar. We know from stratigraphical evidence (above) that at the end of the Oxfordian, 180 m of sandstone and mudstone (density 2.6 g cm⁻³) plus 340 m of mudrock (density 2.4 g cm⁻³) had accumulated, giving a lithostatic load of 128 bar, plus an arbitrary additional 10 bar from seawater pressure in a sediment-starved basin, totalling 138 bar. Thus the earliest time that quartz diagenesis could have taken place was in the late Oxfordian.

Case 2 utilizes an organic geothermometer value of 94 °C (Table 1) implying an overburden pressure of 240 bar from fluid inclusion data. Stratigraphical evidence shows that a lithostatic overburden of 252 bar would have been generated by the end of the Tithonian, plus 10 bar from seawater in a sediment-starved basin, totalling 262 bar. Thus the latest time for quartz diagenesis was during the late Tithonian, the mean of these ages¹⁹ is 150 ± 6 Myr.

To estimate geothermal gradients during quartz diagenesis we must infer sediment overburden thickness at the time²⁰. In both cases we estimate that the middle Jurassic section is increased from 180 m to 200 m, as the reservoir sands at the base must have been compacted to their cemented thickness, and a 10 °C seafloor temperature is assumed. In Case 1, 340 m of mudrock would represent an initial thickness²⁰ of about 500 m, giving a 700 m section and a geothermal gradient of 97 °C km⁻¹. In Case 2, mudrock layers of 340 m, 200 m and 320 m would represent 370 m, 250 m and 500 m respectively²⁰ giving a 1,320 m section and a geothermal gradient of 71 °C km⁻¹. Although these figures are simply guidelines, they do imply a very high geothermal gradient at the end of a short period of very rapid basin subsidence (up to 100 m per Myr). We know from organic geochemistry (Table 1) that the maximum sediment temperature did not exceed 97 °C, and from electric log sonic velocity data that the maximum burial depth

of these sediments was about 3.0 km (present-day thicknesses), so that the geothermal gradient at maximum burial was only $29^{\circ}\text{C km}^{-1}$. The present-day geothermal gradient from borehole measurements is $38^{\circ}\text{C km}^{-1}$. Thus evidence in Beatrice suggests that the geothermal gradient varied through time²¹, with very high temperatures immediately following the most rapid phase of basin subsidence, increasing only slightly, if at all²², during the subsequent Cretaceous thermal subsidence²² of the basin. It may be that rock microfracturing associated with basin rifting and subsidence encouraged rapid circulation of diagenetic fluids.

We have seen that diagenetic quartz forms about 13% of the reservoir sandstones; possible sources of this quartz could be: pressure solution, dissolution of silt-size quartz or of biogenic silica, feldspar breakdown, or illitization of smectite clays. The first three alternatives are inconsistent with petrographic observations. Feldspar breakdown is also insufficient, because even if dissolved feldspar had formed 10% of original grains, silica released could only have contributed 2% of the diagenetic quartz; thus we are left with a quartz supply from the illitization reaction^{23–25}. This reaction occurs at about $70\text{--}90^{\circ}\text{C}$ ^{23–25}, a similar temperature to that recorded by the fluid inclusions. In addition, Pearson *et al.*²⁶ have argued that smectite-rich muds were present in the Inner Moray Firth. Thus it seems probable that illitization in surrounding mudrocks slightly deeper in the half graben¹⁸ released silica-rich fluids which travelled up-dip through permeable sandstone beds, and authigenic quartz was precipitated as the aqueous fluids cooled beneath mudstone roofs—with a fall in temperature from 100°C to 80°C the solubility of quartz decreases by 56%⁷.

Thus diagenetic quartz growth occurred at several times at temperatures between 68°C and 94°C and pressures of 100–240 bar. Assuming that lithostatic pressure is achieved at all depths greater than 1.5 km the quartz grew in a very high geothermal gradient of 71 to $97^{\circ}\text{C km}^{-1}$, immediately following episodes of rapid basin subsidence during the Oxfordian and Tithonian. Sediments reached their maximum temperatures near the end of this rifting and the geothermal gradient decreased sympathetically with post-rifting subsidence. Silica was released by illitization of smectite clays and precipitated as quartz in permeable sandstone aquifers when the transporting aqueous solutions cooled.

I. M. S. was funded by the NERC and the fluid inclusion equipment supplied through NERC grant GR3/3726A. We thank M. J. Russell (University of Strathclyde) for suggesting examination of fluid inclusions, R. Archer and J. Sargeant of Britoil for helpful discussions, and Britoil and Beatrice partners for permission to publish. We thank A. S. Mackenzie for a constructive review.

Received 23 September; accepted 17 October 1983.

1. Pryor, W. A. *Bull. Am. Ass. Petrol. Geol.* **57** 162–189 (1973).
2. Hayes J. B. *Soc. Econ. Paleont. Mineral. Spec. Publ.* **26**, 127–139 (1979).
3. Linsley, P. N., Potter, H. C., McNab, G. & Racher, D. *Mem. Am. Ass. Petrol. Geol.* **30**, 117–130 (1980).
4. Haszeldine, R. S., Samson, I. M. & Cornford, C. *Clays Clay Miner.* (submitted).
5. Loucks, R. G., Babout, D. G. & Galloway, W. E. *Trans. Gulf-Cst. Ass. geol. Soc.* **27**, 109–120 (1977).
6. Curtis, C. D. in *Geochemistry and Exploration of Europe* (ed. Brooks, J.) 113–125, (Blackwells, London, 1983).
7. Holland, H. D. & Malinin, S. D. in *Geochemistry of Hydrothermal Ore Deposits* 2nd Edn (ed. Barnes, H. L.) 461–508 (Wiley, New York, 1979).
8. Fournier, R. O. & Marshall, W. L. *Geochim. cosmochim. Acta* **47**, 587–596 (1983).
9. Burruss, R. C. *Am. J. Sci.* **281**, 1104–1126 (1981).
10. Crawford, M. L. in *Fluid Inclusions: Applications to Petrology* (eds Hollister, L. S. & Crawford, M. L.) *Min. Ass. Canada Short Course, Handbk.* **6**, 75–100 (1981).
11. Potter, R. W. II, Clyne, M. A. & Brown, D. C. *Econ. geol.* **73**, 284–285 (1978).
12. Cornford, C., Morrow, J. A., Turrington, A., Miles, J. A., & Brooks, J. in *Petroleum Geochemistry and Exploration of Europe* (ed. Brooks, J.) 175–194 (Academic, London, 1983).
13. Mackenzie, A. S. & Maxwell, J. G. in *Organic Maturation Studies and Fossil Fuel Exploration* (ed. Brooks, J.), 239–254 (Academic, London, 1981).
14. McKenzie, D., Mackenzie, A. S., Maxwell, J. R. & Sajgo, C. *Nature* **301**, 504–506 (1983).
15. Potter, R. W. II & Brown, D. C. *Bull. U.S. geol. Surv.* **1421-C** (1977).
16. Haas, J. L. Jr *Bull. U.S. geol. Surv.* **1421-A** (1976).
17. Fyfe, W. S., Price, N. J. & Thomson, A. B. *Fluids in the Earth's Crust* (Elsevier, Amsterdam, 1977).
18. Cheshier, J. A. & Bacon, M. *Inst. geol. Sci. Rep.* 75/11 (HMSO, London 1975).
19. Harland, W. B. *et al. A Geologic Timescale* (Cambridge University Press, 1982).

20. Perrier, R. & Quiblier, J. *Bull. Am. Ass. Petrol. Geol.* **58**, 507–520 (1974).
21. Price, L. C. *J. Petrol. Geol.* **6**, 5–38 (1983).
22. McKenzie, D. *Earth planet. Sci. Lett.* **55**, 87–98 (1981).
23. Hower, J., Eslinger, E. V., Hower, M. E. & Perry, E. A. *Bull. geol. Soc. Am.* **87**, 725–737 (1976).
24. Boles, J. R. & Franks, S. G. *J. sedim. Petrol.* **49**, 55–70 (1979).
25. Dypvik, H. *Bull. Am. Ass. Petrol. Geol.* **67**, 160–165 (1983).
26. Pearson, M. J., Watkins, D. & Small, J. S. *Develop. Sedimentol.* **35**, 665–675 (Elsevier, Amsterdam 1982).

Did the Dalradian slides originate as extensional faults?

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Rocks of the Dalradian Supergroup^{1,2} of Scotland and Ireland were deposited in late Proterozoic–early Cambrian times on what became the southern margin of the Laurentian plate, and underwent metamorphism and deformation when this margin was affected by the Grampian orogeny in the late Cambrian^{3,4}. The resulting complex structure is still not fully understood, and a continuing problem is presented by the origin and significance of the ‘slides’, a series of ductile faults which lie sub-parallel to bedding⁵. Although numerous slides have been recognized within the Dalradian terrain⁶ the term has seldom been used outside the British Caledonides. It is generally assumed that these slides are synmetamorphic and were formed during Grampian deformation. However, a consideration of their geometry, stratigraphic relationships and context now suggests that they may have been initiated as syndepositional extensional faults which were only modified, and not formed, during the Grampian orogeny.

The numerous local stratigraphic successions recognized in the Scottish Dalradian have been consolidated into a fourfold sequence comprising the Grampian (oldest), Appin, Argyll and Southern Highland Groups¹. These have a combined stratal thickness of some 25 km and the Dalradian thus represents one of the major supracrustal sequences of the Caledonides². It was deposited on a basement composed of crystalline gneisses (Lewisian) and a cover sequence (Moine) which had undergone orogenic reworking during the Grenville cycle (~1,000 Myr). Much of the Dalradian is characterized by distinctive lithologies, facilitating subdivision and correlation, and leading in turn to the recognition of many stratigraphic and structural complexities. One such is the problem posed by the ‘slides’. This term was introduced by Bailey in 1910⁷ for planes along which parts of the stratigraphic succession in the Ballachulish district had undergone thinning or complete excision. Slides were subsequently widely mapped within the Dalradian terrain (Fig 1) and in the Moine and Lewisian rocks of the North-West Highlands⁸. A recent tendency has been to expand the usage, inadvisably in our view, to include platy high strain zones without demonstrable stratigraphic separation^{9,10}.

Grampian structure is characterized by large, often recumbent primary folds which face north-west and south-east away from a central zone. Current interpretations involve upward and outward expulsion of material from this zone, the so-called ‘fountain of nappes’ hypothesis^{11,12}. Attempts to integrate the slides into this scheme by interpreting them as ductile thrusts associated with the primary folding have not been successful (Fig. 2). As Bailey¹³ was well aware, important slides such as Ballachulish and Fort William (Fig. 1) emplace younger rocks over older and thus have the geometry of ‘lags’ not thrusts. In

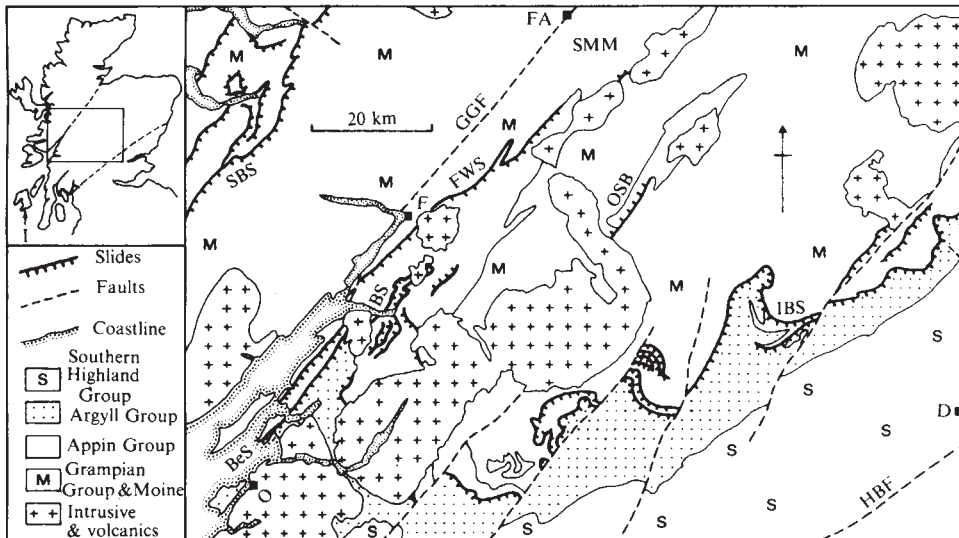


Fig. 1 Simplified geological map of part of the southwestern Highlands of Scotland showing some of the slides in the Dalradian and Moine: BeS, Benderloch slide; BS, Ballochulish slide; FWS, Fort William slide; IBS, Iltay Boundary slide; SBS, Sgurr Beag slide. Ticks are on down-dip side of slides. Box inset shows location of map. Towns: D, Dunkeld; F, Fort William; FA, Fort Augustus; O, Oban. Other localities: I, Islay; OSB, Ossian steep belt; SMM, southwestern Monadhliath Mountains. Faults: GGF, Great Glen Fault; HBF, Highland Boundary Fault. Compiled from refs 12, 27, 37–39.

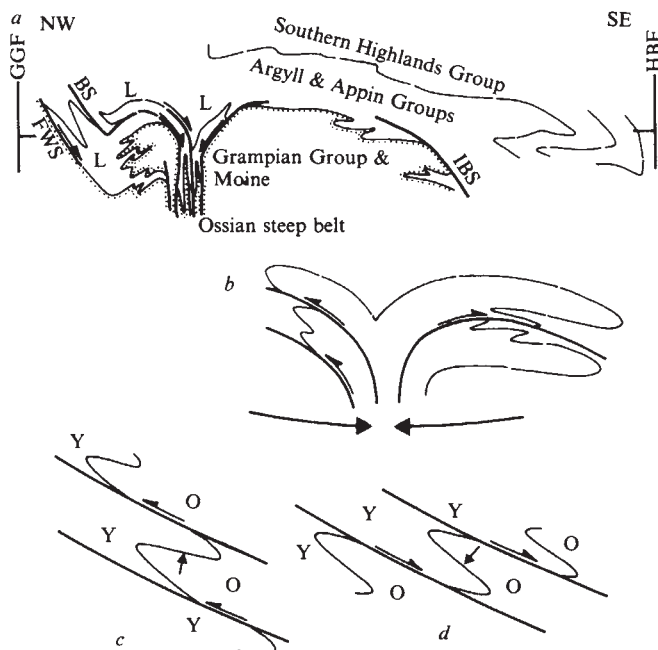


Fig. 2 *a*, Diagrammatic profile across the Grampians¹², between Fort Augustus and Dunkeld (Fig. 1). Major slides are shown by bold lines, with their displacement directions inferred from the stratigraphy indicated by half arrows and labelled as in Fig. 1. GGF, Great Glen Fault; HBF, Highland Boundary Fault; L, Lochaber Sub-group. *b*, The 'fountain of nappes' hypothesis¹² showing slide displacements opposite in sense to those shown in *a*. *c*, Lag or extensional fault geometry, as in *a*. *d*, Contractional thrust geometry, as in *b*. Y and O refer to younger and older beds respectively.

addition, associated folds often show attenuation of their right-way-up, not their inverted limbs (Fig. 2).

The concept of a lag as a thrust plane on which the upper plate was displaced less far forward than the lower seems improbable and the term is now rarely used. Lag geometry is in fact extensional, not contractional. We propose a radical solution to the problem illustrated in Fig. 2 by suggesting that the slides which show extensional geometry were initiated during Dalradian sedimentation as growth faults, not during the Grampian orogeny as thrusts.

During the late Proterozoic the Dalradian terrain evolved from a slowly subsiding stable shelf on which sediments of the Grampian and Appin Groups were deposited, into a series of rapidly subsiding fault-bounded basins (Argyll Group). This

increasing instability is thought to have reflected an increasing rate of crustal stretching which eventually led, at about the beginning of the Cambrian, to rupture of the Proterozoic Supercontinent¹⁴ and birth of the Iapetus Ocean¹⁵. The time of rupture was marked by the extrusion of the tholeiitic Tayvallich volcanics¹⁶ although this volcanism appears to have been peripheral to the site of continental splitting which lay further to the south-east. It is now considered that during Argyll Group times the Dalradian terrain of the southwestern Highlands developed into a type of quasi-oceanic crust similar to that now forming at the head of the Gulf of California¹⁷. In this, development of a mature spreading centre is suppressed by the rapid influx of sediment and spreading takes place on a limited scale by sill and dyke injection. Thus, it is thought that there was an attempt to form oceanic crust within the present area of Dalradian outcrop, but that this was aborted and the final split took place further to the south-east.

Dalradian deposition can be viewed in the context of the McKenzie model for basin subsidence by lithosphere stretching and thermal relaxation^{18–20}. During Appin Group times stretching and thinning was insufficient to allow volcanic activity, and sedimentation was able to keep pace with subsidence. More rapid stretching produced the deep Argyll Group basins^{21,22}. By end-Argyll times the sediment pile had attained a maximum thickness of some 12 km and the lithosphere had thinned by a factor of about four, permitting the injection of basic magma towards the top of the sediment column. An interpretation of the lateral thickness and facies changes within the Argyll Group suggests that this stretching was accomplished by major syn-depositional faults, with throws of up to several kilometres and an across-strike spacing of tens of kilometres, dividing the area into blocks and basins²². Current ideas on extensional faulting suggest that such faults can often be listric in form, flattening out at a depth of 8–12 km^{19,20,23,24}. This would be the depth of the lower part of the Appin Group by mid-Argyll times. Figure 3 illustrates these faults schematically; some of them we suggest are now represented by slides. Later subsidence to accommodate the Southern Highland Group may have been largely thermal, following abandonment of the incipient rifting centre, no further extension being required.

It has always been assumed that the slides are of Grampian age, associated with the primary deformation. Many carry a penetrative fabric and some coincide with a change in facies of early folds^{6,25,26}. These must either have been initiated during the Grampian deformation or, if earlier, suffered reactivation. On the other hand the Benderloch slide, for example, has the regional fabric superimposed obliquely across it²⁷ and appears to be pre-Grampian. As Litherland²⁸ pointed out, the slide coincides with a major facies change in the Argyll Group which thickens rapidly across it to the south-east. Our contention is

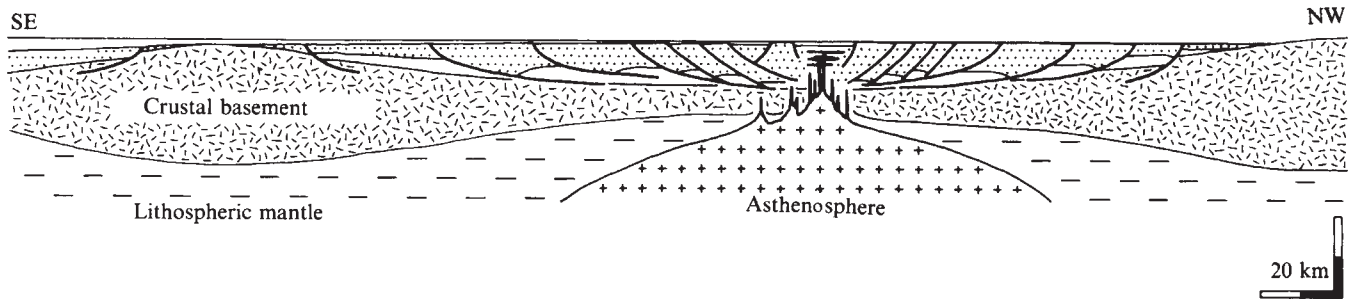


Fig. 3 Sketch illustrating the crustal structure of the Dalradian terrain, in late Argyll Group times, showing listric and other normal faults. Continental crust is on the verge of rupturing with the formation of quasi-oceanic crust by sill and dyke emplacement. Crustal stretching subsequently ceased here, the complete failure of the Laurentian-Baltic plate taking place at another site of crustal attenuation to the south-east of the area shown on this diagram. Ornament for Appin and Argyll Groups is as shown on Fig. 1. Older basement rocks are not differentiated.

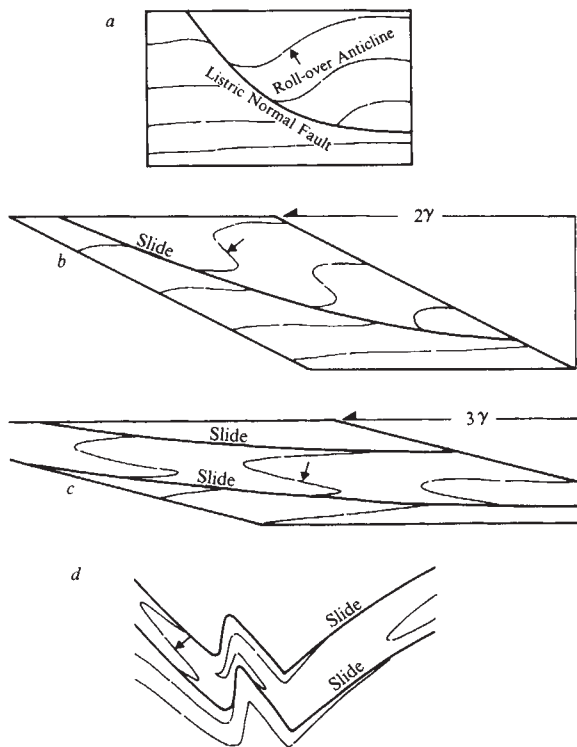


Fig. 4 Diagrams illustrating the geometrical possibility of deriving Grampian slide and primary fold geometry by the superimposition of simple shear strain on early extensional structures. *a*, Listric normal fault and associated roll-over anticline²⁹. *b*, Passive rotation of slide plane and bedding under a shear strain of 2γ . *c*, Under 3γ 'primary' recumbent folds are produced. Two slide zones are shown. *d*, Secondary refolding of *c* produces a profile comparable to that of the southwestern Monadhliath Mountains (compare ref. 30, Fig. 3).

that slides such as the Benderloch, which emplace younger rocks over older and thus have extensional geometry, started life as listric growth faults and either behaved entirely passively during Grampian deformation, or were reactivated. Studies are needed to test whether displacements inferred from fabrics and folds associated with particular slides are compatible with the displacements required by the stratigraphy.

Figure 4 illustrates that the hypothesis is geometrically realistic, and can also account for those associated folds which have their 'wrong' limb attenuated. We start with a listric normal fault and roll-over anticline, as seen in many examples revealed by reflection profiling of extensional continental margins²⁹. Accepting the view that Grampian deformation in Scotland did involve the expulsion of material north-west and south-east from a central zone (which coincided approximately with a major depocentre) we model the deformation as simple shear

with an opposite sense of displacement to that of the growth fault. Passive rotation of the material planes (bedding and fault) under quite modest shear strains produces folds with their right-way-up limbs apparently attenuated at the slide. Secondary refolding generates a geometry comparable with that of the published profile of the southwestern Monadhliath Mountains^{30,31}.

Our hypothesis could throw light on unexplained features of Highland geology. For example the Ossian steep belt¹² (Fig. 2) could represent a highly compressed graben. The Loch Skerrols 'thrust' in Islay³² marks the western margin of the Grampian orogenic belt against the north-west foreland, a position analogous to that of the Moine thrust zone to the north, a major contractional structure. However, the Loch Skerrols displacement brings Argyll Group rocks in the hanging wall against older Bowmore sandstone and thus appears to be extensional. It may have developed on the site of a major basin-margin fault.

Structures referable to the major phase of extension which affected the sub-Dalradian basement in the late Proterozoic, and which might have survived Grampian-Caledonian overprinting, should be sought. Extensional displacements at depth would generate ductile fabrics difficult to distinguish from those associated with subsequent compressional events. The reactivation of syndepositional faults in compression may well represent an important mechanism of upper lithosphere shortening in zones of continental convergence³³.

Finally, we point to the apparent coincidence in time between the Moravian pegmatite 'event' which affected the Old Moine basement at around 750 Myr^{9,34}, and the onset of Grampian-Dalradian sedimentation. The traditional interpretation of the Moravian as a late Grenville, pre-Grampian orogenic event (equivalent to the now-discredited Carolinides of north-east Greenland³⁵) is difficult to sustain without clear evidence of compressional structures or syntectonic metamorphism. Although it has been proposed that these pegmatites are associated with ductile shear zones⁹, they are clearly dilational and lack synkinematic crystallization fabrics³⁶. We suggest that they were formed during a major phase of crustal stretching and enhanced heat flow associated with the onset of cover sedimentation.

Received 10 August; accepted 31 October 1983.

- Harris, A. L. & Pitcher, W. S. *Geol. Soc. Lond. Spec. Rep.* No. 6, 52-75 (1975).
- Harris, A. L., Baldwin, C. T., Bradbury, H. J., Johnson, H. D. & Smith, R. A. *Geol. J. Spec. Issue* No. 10, 115-138 (1978).
- Lambert, R. St. J. & McKerrow, W. S. *Scott. J. Geol.* **12**, 271-292 (1976).
- Bradbury, H. J., Smith, R. A. & Harris, A. L. *J. geol. Soc. Lond.* **132**, 677-684 (1976).
- Fleuty, M. J. *Geol. Mag.* **101**, 452-456 (1964).
- Hutton, D. W. H. *Earth Sci. Rev.* **15**, 151-172 (1979); *Geol. Soc. Lond. Spec. Publ.* No. 9, 261-265 (1981).
- Bailey, E. B. Q. *J. geol. Soc. Lond.* **66**, 586-608 (1910).
- Tanner, P. W. G. *J. geol. Soc. Lond.* **126**, 435-463 (1971).
- Piasecki, M. A. J. & van Breemen, O. *Nature* **278**, 734-736 (1979); *Trans. R. Soc. Edinburgh: Earth Sci.* **73**, 119-134 (1983).
- Mendum, J. R. *Geol. Soc. Lond. Spec. Publ.* No. 8, 291-297 (1979).
- Roberts, J. L. *J. geol. Soc. Lond.* **130**, 93-124 (1974).
- Thomas, P. R. *Geol. Soc. Lond. Spec. Publ.* No. 8, 205-211 (1979).
- Bailey, E. B. & Maufe, M. A. *Mem. Geol. Surv. Scotl.* **113** (1960).
- Piper, J. D. A. *Earth planet. Sci. Lett.* **59**, 61-89 (1982).
- Anderton, R. J. *J. geol. Soc. Lond.* **139**, 421-431 (1982).
- Graham, C. M. *J. geol. Soc. Lond.* **132**, 61-84 (1976).

17. Moore, D. G. *Bull. geol. Soc. Am.* **84**, 1883-1906 (1973).
18. McKenzie, D. *Earth planet. Sci. Lett.* **40**, 25-32 (1978).
19. Le Pichon, X. & Sibuet, J.-C. *J. geophys. Res.* **86B**, 3708-3720 (1981).
20. Dewey, J. F. *J. geol. Soc. Lond.* **139**, 371-412 (1982).
21. Knill, J. L. in *The British Caledonides* (eds Johnson, M. R. W. & Stewart, F. H.) 99-121 (Oliver & Boyd, Edinburgh, 1963).
22. Anderton, R. *Geol. Soc. Lond. Spec. Publ.* No. 8, 483-488 (1979).
23. Profett, J. M. *Bull. geol. Soc. Am.* **89**, 1389-1403 (1978).
24. Montadert, L., Roberts, D. G. & De Charpal, O. *Init. Rep. DSDP Leg 48*, 1025-1060 (1979).
25. Sanderson, D. J., Andres, J. R., Phillips, W. E. A. & Hutton, D. W. H. *J. geol. Soc. Lond.* **137**, 289-302 (1980).
26. Hutton, D. W. H. *Trans. R. Soc. Edinburgh: Earth Sci.* **73**, 151-171 (1983).
27. Litherland, M. *Scott. J. Geol.* **18**, 205-225 (1982).
28. Litherland, M. *Scott. J. Geol.* **16**, 105-123 (1980).
29. Wernicke, B. & Burchfiel, B. C. *J. struct. Geol.* **4**, 105-115 (1982).
30. Haselock, P. J., Winchester, J. A. & Wittles, K. H. *Scott. J. Geol.* **18**, 275-290 (1982).
31. Roberts, J. L. & Treagus, J. E. *Scott. J. Geol.* **13**, 237-254 (1977).
32. Westbrook, G. K. & Barradaile, G. J. *Scott. J. Geol.* **14**, 213-224 (1978).
33. Jackson, J. A. *Nature* **283**, 343-346 (1980).
34. van Breemen, O., Halliday, A. N., Johnson, M. R. W. & Bowes, D. R. *Geol. J. Spec. Iss.* No. 10, 81-106 (1978).
35. Jepsen, H. F. & Kalsbeek, F. *Grønlands geol. Unders. Rapp.* **106**, 7-14 (1981).
36. Powell, D., Brook, M. & Baird, A. W. *J. geol. Soc. Lond.* **140**, 813-832 (1983).
37. Roberts, J. L. & Treagus, J. E. *Scott. J. Geol.* **13**, 165-184 (1977); *Geol. Soc. Lond. Spec. Publ.* No. 8, 199-204 (1979).
38. Hickman, A. H. *Scott. J. Geol.* **14**, 191-212 (1978).
39. Powell, D. *J. geol. Soc. Lond.* **130**, 577-593 (1974).

The gradual decline of the dinosaurs—fact or fallacy?

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Both great dinosaurian orders (Saurischia and Ornithischia) were major components of terrestrial vertebrate faunas for ~150 Myr. The prevailing view is that dinosaurs attained an evolutionary acme late in the Cretaceous, after which they gradually declined in taxonomic diversity over ~10 Myr to their extinction 63 Myr ago¹⁻⁶. The postulated decline is usually supported by comparing diversity levels in 76 Myr-old and 64 Myr-old dinosaurian assemblages from North America. The resulting differences in diversity have never been compared, however, with those observed between older dinosaurian assemblages, when their extinction was not imminent. I show here that, taken as a whole, the known fossil record of North American dinosaurs shows no evidence of a decline in taxonomic diversity lasting several million years or more before their extinction.

The fossil record of dinosaurs preserved in Triassic to Cretaceous strata in North America is unsurpassed on other continents in terms of the number of specimens that have been recovered, and the degree to which they are representative of successive phases of dinosaurian evolution⁷. Thus, a possible terminal Cretaceous decline in diversity is assessed here through a compilation of all of the known families and genera of North American dinosaurs⁸ (see Table 1 and Fig. 1). The data are based on skeletal materials collected largely from the eastern Rocky Mountains and adjacent plains, and from sediments associated with the Newark Rift System extending from Nova Scotia to North Carolina. Only late Triassic, late Jurassic and late Cretaceous terrestrial reptilian faunas have been relatively well sampled. These circumstances, together with the fact that many available collections are inadequately studied, imply that fewer than one-half of the dinosaurian genera which at one time or another were indigenous to North America have been recorded. Nevertheless, the compilation suggests that dinosaurs increased in diversity through the Mesozoic era. Data based on mammalian material collected from the same lithostratigraphic units⁹ show similar trends (the differences in slopes of linear regressions for dinosaurian and mammalian genera and families against time are not statistically significant). Dinosaurs show no more evidence of a late Mesozoic decline than do mammals.

Rarefaction analyses^{10,11} should be carried out on dinosaurian (and contemporary mammalian) assemblages to remove the

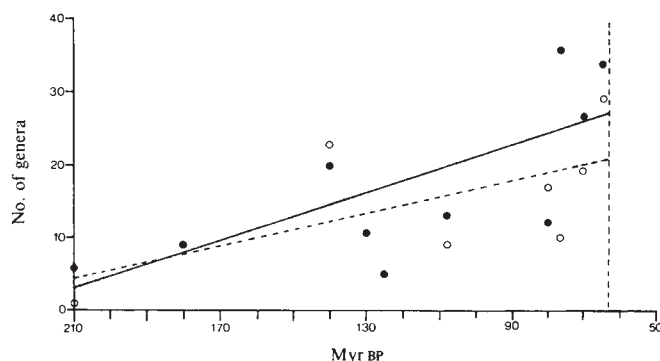


Fig. 1 Generic diversity of North American dinosaurs (closed circles) and mammals (open circles) plotted against time measured in Myr BP. The regression line for dinosaurs (solid line) is $g = 37.1 - 0.16t$, $r = -0.71$, and for mammals (broken line) $g = 27.2 - 0.11t$, $r = -0.61$, where g = number of genera and t = time in Myr. The vertical broken line represents the Cretaceous-Tertiary boundary plotted at 63 Myr BP.

effect of sample size on observed diversity. This can seldom be done because of the lack of available data. It is possible, however, in the case of the most diverse dinosaurian assemblage known, that occurring in sediments deposited 13 Myr before the end of the Cretaceous in the vicinity of Dinosaur Provincial Park, Alberta. This locality has also yielded the largest number of dinosaurian skeletal fragments known from any area of badlands of comparable size¹²⁻¹⁴. A rarefaction trend (Fig. 2) was plotted, based on the number and proportions of generically identified, articulated specimens from the Alberta locality.

Collections of dinosaur skeletal materials from younger assemblages are smaller¹⁵ and less well studied. The diversity contained within one relatively well studied collection of 20 specimens in the Los Angeles County Museum, from sediments deposited in eastern Montana ~1 Myr before the extinctions¹⁶⁻²⁰, falls within the 95% confidence interval for the rarefaction curve calculated for the older Canadian site (asterisk, Fig. 2). This is despite the fact that the older locality has been shown to contain an ecological gradient and therefore may have yielded a more than usually diverse assemblage¹²⁻¹⁴. Diversity levels were evidently maintained in the terminal Cretaceous sample from Montana despite environmental changes in the wake of a major retreat of the midcontinental sea which occurred ~1 Myr previously^{21,22}, but did not recur at the end of the dinosaurian era²³. The diversity contained within another small collection of a more inland facies from the Scollard Formation of Alberta^{24,25} (with apparently 7 genera distributed among 17 specimens) may be similar to that of the Montana sample. No other assemblage of terminal Cretaceous dinosaurs is sufficiently well studied to serve as a useful basis for comparison. The

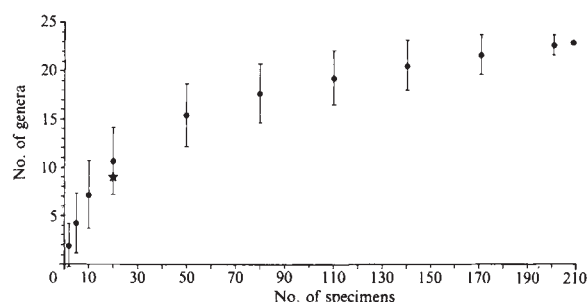


Fig. 2 Rarefaction curve plotted for generically identified dinosaur specimens from Dinosaur Provincial Park, Alberta, dating from ~13 Myr before the terminal Cretaceous extinctions. The asterisk indicates the position of the Hell Creek Fossil Field subsample in the collections of the Los Angeles County Museum dating from ~1 Myr before the terminal Cretaceous extinctions.

Table 1 Total numbers of dinosaurian and mammalian genera and families identified in Mesozoic sediments of North America

Marine stage (continental stage)	Absolute age	No. of genera		No. of families	
		Dinosaurs	Mammals	Dinosaurs	Mammals
Upper Maastrichtian (Lancian)	~64	34	29	19	16
Upper Campanian-Lower Maastrichtian (Edmontonian)	~70	27	19	13	13
Upper Campanian (Judithian)	~76	36	10	15	8
Lower Campanian (Aquilian)	~80	12	17	8	11
Aptian-Albian (Paluxian)	~108	13	9	10	7
Valanginian	~125	5	—	5	—
Kimmeridgian-Portlandian	~140	20	23	13	8
Hettangian-Pleinsbachian	~180	9	—	7	—
Carnian	~210	6	1	5	1

The absolute age is given in Myr BP. Mammalian generic diversity levels are probably exaggerated for Kimmeridgian-Portlandian time due to the assignment of separate names to disassociated upper and lower dentitions³⁹.

Cloverly assemblage from Wyoming and Montana²⁶⁻²⁹, which predates the end of the Cretaceous by ~45 Myr and contains 6 genera distributed among at least 50 specimens, is evidently of low diversity for reasons other than its proximity in time to the Cretaceous-Tertiary boundary.

The record of dinosaurian diversity through Mesozoic time in other parts of the world is less complete. A sequence of continental strata in Mongolia, although not well dated, is clearly of late Cretaceous age³⁰⁻³². A succession of three dinosaurian assemblages in these sediments shows no evidence of a decline in diversity³⁰. Other potentially excellent continental records³³⁻³⁵ will require more study before broad trends in dinosaurian diversity become apparent. It is clear, however, that dinosaurs were distributed around the world during terminal Cretaceous time³⁶.

This compilation of dinosaurian families and genera from Mesozoic strata in North America seems to be in general conformity with contemporary diversity trends seen in terrestrial mammals, marine invertebrates³⁷ and terrestrial plants³⁸. There is no compelling evidence for a terminal Cretaceous decline in dinosaurian diversity on a time scale of millions of years.

I thank F. Asaro for suggesting that a compilation similar to that of Table 1 be made and D. M. Raup for the rarefaction analysis shown in Fig. 2. I also thank W. Alvarez, P. J. Currie, P. Dodson, J. S. McIntosh and K. A. Pirozynski for critically reviewing the manuscript. D. Baird, M. K. Brett-Surman, K. Carpenter, P. Dodson, P. M. Galton, J. R. Horner, W. Langston Jr, R. A. Long and J. S. McIntosh generously provided information on the affinities of numerous dinosaurian specimens.

Received 9 August; accepted 28 October 1983.

- Archibald, J. D. & Clemens, W. A. *Am. Sci.* **70**, 377-385 (1982).
- Bakker, R. T. in *Patterns of Evolution as Illustrated by the Fossil Record* (ed. Hallam, A.) 439-468 (Elsevier, Amsterdam, 1977).
- Buffetaut, E. *Mém. Soc. géol. Fr.* **139**, 47-52 (1980).
- Schopf, T. J. M. *Geol. Soc. Am. spec. Pap.* **190**, 415-422 (1982).
- Rozhdestvensky, A. K. in *Evolution and Change of the Organic Realm at the Mesozoic-Cenozoic Boundary* (in Russian) (ed. Menner, V. V.) 57-82 (Academy of Sciences of the USSR, Moscow, 1978).
- Van Valen, L. & Sloan, R. E. *Evol. Theory* **2**, 37-64 (1977).
- Colbert, E. H. *Dinosaurs, their Discovery and their World*, 300 (Dutton, New York, 1961).
- Russell, D. A. *Sylogosus* (in the press).
- Clemens, W. A., Lillegraven, J. A., Lindsay, E. H. & Simpson, G. G. in *Mesozoic Mammals* (eds Lillegraven, J. A. et al.) 7-58 (University of California Press, Berkeley, 1979).
- Raup, D. M. *Paleobiology* **1**, 333-342 (1975).
- Tipper, J. C. *Paleobiology* **5**, 423-434 (1979).
- Beland, P. & Russell, D. A. *Can. J. Earth Sci.* **15**, 1012-1024 (1978).
- Russell, D. A. *Terra* **21**, 3-9 (1983).
- Dodson, P. *Mosasaurs* **1**, 89-118 (1983).
- Russell, D. A. *Geol. Ass. Canada spec. Pap.* **13**, 119-136 (1975).
- Galton, P. M. & Sues, H. D. *Can. J. Earth Sci.* **20**, 462-472 (1983).
- Molnar, R. E. *J. Paleont.* **52**, 73-82 (1978).
- Molnar, R. E. *J. Paleont.* **54**, 102-108 (1980).
- Morris, W. J. *Los Angeles County Mus. Contr. Sci.* **193**, 1-14 (1970).
- Morris, W. J. in *Athlon, Essays on Palaeontology in Honour of Loris Shano Russell* (ed. Churcher, C. S.) 93-113 (Royal Ontario Museum, Toronto, 1976).
- Gill, J. R. & Cobban, W. A. *U.S. geol. Surv. Prof. Pap.* **776** (1973).
- Jeletzky, J. A. *Geol. Surv. Canada Pap.* **77-18** (1978).
- Frye, C. I. *Bull. N. Dak. geol. Surv.* **54**, 65 (1969).
- Sternberg, C. M. *Geol. Surv. Canada Pap.* **47-1** (1947).
- Russell, D. A. *Natn. Mus. Canada nat. Hist. Pap.* **36** (1967).
- Ostrom, J. H. *Bull. Peabody Mus. nat. Hist.* **30**, 165 (1969).
- Ostrom, J. H. *Bull. Peabody Mus. nat. Hist.* **35**, 234 (1970).

- Ostrom, J. H. *Mus. Comp. Zool. Breviora* **439** (1976).
- Sues, H. D. *Palaeontographica A* **169**, 51-72 (1980).
- Osmolska, H. *Mém. Soc. géol. Fr.* **139**, 145-150 (1980).
- Fox, R. C. *Geol. Ass. Canada spec. Pap.* **18**, 577-594 (1978).
- Kielan-Jaworowska, Z. & Sloan, R. E. *Acta palaeont. pol.* **24**, 187-197 (1979).
- Bonaparte, J. F. *El Mesozoico de America del Sur y sus Tetrapodes* (Fundacion Miguel Lillo, Tucuman, 1978).
- Taquet, P. *Mém. Soc. géol. Fr.* **139** (1980).
- Jacobs, L. L. (ed.) *Aspects of Vertebrate History*, 210 (Museum of Northern Arizona Press, Flagstaff, 1980).
- Russell, D. A. *Geol. Soc. Am. spec. Pap.* **190**, 401-405 (1982).
- Sepkoski, J. J. Jr, Bambach, R. K., Raup, D. M. & Valentine, J. W. *Nature* **293**, 435-437 (1981).
- Knoll, A. H., Niklas, K. J. & Tiffney, B. H. *Science* **206**, 1400-1402 (1979).
- Prothero, D. R. *Bull. Am. Mus. nat. Hist.* **167**, 277-325 (1981).

Courtship feeding increases female reproductive success in bushcrickets

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Parental investment theory suggests that investment by males through courtship feeding of their mates may represent an important source of nutrition which increases female fitness and ultimately influences patterns of sexual selection^{1,2}. In certain insects males provide nutritional products from reproductive accessory glands during mating; these are either eaten by the female or are absorbed in her genital tract^{2,3}. Male bushcrickets (Orthoptera: Tettigoniidae) feed their mates with an elaborate spermatophore consisting of a spermatophylax, which is eaten by the female after mating, and a sperm ampulla, eaten after the ejaculate has emptied^{4,5}. Studies of bushcricket mating systems have indicated that spermatophore nutrients are important to females—females prefer to mate with males able to supply larger spermatophores⁶—and field studies of species with very large spermatophores have revealed a role-reversal in reproductive behaviour, with females aggressively competing for males capable of producing spermatophores^{7,8}. Although radiolabelling experiments (with several insect species) have demonstrated that male-derived nutrients are incorporated into eggs^{3,9-11}, these studies do not demonstrate that this sort of courtship feeding enhances female fitness. Here I report the results of an experiment which shows that feeding on the spermatophore enhances the reproductive success of female bushcrickets (*Requena verticalis* Walker) by increasing the numbers and size of eggs produced.

In *R. verticalis*, radiolabelled nutrients from the spermatophore are incorporated to a large degree into maturing eggs⁵. A previous study of this species showed no influence of the amount of male-derived nutrition on fecundity⁵. All females

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Table 1 Effect of number of spermatophylaxes eaten on egg number

No. of spermatophylaxes eaten	0	1	3	7		
No. of eggs	31.9±2.5	45.8±3.1	59.8±5.1	70.7±4.7		
($\bar{x}$ +s.e.)	(32.9)*	(46.3)*	(59.7)*	(69.3)*		
Sample size	14	12	12	13		
Analysis of variance						
	Slope	SS	d.f.	MS	F	P
Treatment		9,274.8	3	3,091.6	23.3	<0.001
Covariates:						
Day mated	-0.56	1,513.8	1	1,513.8	11.4	0.002
Pronotum length	9.5	1,089.2	1	1,089.2	8.2	0.006
Explained		14,683.9	5	2,936.8	22.2	<0.001
Residual		5,966.0	45	132.6		

Subjects for the experiment were collected as late instar females in Perth, Western Australia, and reared to adulthood in the laboratory (14 h light:10 h dark at 25°C). Nymphs were all fed on a diet of rolled oats, tropical fish food and pollen (17% crude protein) and fresh carrot. Adult females were measured (pronotum length in mm (± 0.01 mm)) and mated 7–15 days after the adult moult, over a 41-day period in February–March 1983. For treatment 0, the intact spermatophylax was removed from the ampulla with forceps before the female grasped it (females remove the spermatophylax from the ampulla a few minutes after mating³). The female was then placed in a glass tube for 4–5 h (sufficient time to allow sperm supplies to empty) to prevent her from removing the sperm ampulla prematurely². After releasing the female from the tube, I removed the empty ampulla before she could consume it. Additional spermatophylaxes for treatments 3 and 7 were obtained from both treatment 0 matings and other matings set up at the same time. These spermatophylaxes were fed to females within an hour of being produced. Spermatophylaxes received by each female from treatments 3 and 7 were derived from different males. Females from all matings were housed in 1-l jars placed over the oviposition substrate (dry sand) and fed on rolled oats (12% crude protein) and fresh carrot. Eggs were collected from the sand at 7, 14, 21, 28 and 30 days post-mating. Dissection of females revealed that they all had extensive fat body in the abdominal cavity. SS, sum of squares; MS, mean of squares; d.f., degrees of freedom.

* Mean adjusted for the effects of covariates, day mated and pronotum length.

in that study received a high protein diet, with some females receiving one extra spermatophylax at mating. For bushcrickets, limited access to high protein food sources (several sub-families)^{12,16} and up to 12 matings, and thus spermatophores, per female (*Anabrus simplex*)¹⁶ have been documented in nature. Therefore, in the present study females were given a wide range of the male nutrient contribution and were maintained on a lower (12%) protein diet.

Four treatments were established in which the amount of male-derived nutrition was manipulated but the amount of sperm and other ejaculatory materials received by females was controlled. Individuals were all mated once and all matings were conducted over a 41-day period. Treatment numbers represent the number of spermatophylaxes females consumed: hence, treatment 0, the intact spermatophylax was removed after mating and the empty ampulla after insemination (that is, females received no oral nutrition from the male); treatment 1, normal matings in which females were allowed to consume the spermatophylax and ampulla; treatment 3, females were fed two additional spermatophylaxes, one every 6 or 7 days, during the first 14 days after a normal mating; and treatment 7, females were fed six additional spermatophylaxes, one every 2 or 3 days, during the first 14 days following a normal mating. All individuals were allowed to oviposit in dry sand for 30 days after mating. This time period was sufficient to allow the incorporation of a large amount of male-derived nutrition into mature eggs³. Females were then killed, numbers of mature ovarian eggs (those with a chorion) were added to the counts of eggs laid and the spermathecae were checked for the presence of sperm. Of 54 individuals, 51 had ample sperm supplies; the three females without sperm were discarded from the analysis (additional details of methods are presented in Table 1 legend).

Analysis of variance of egg number by treatment with size (pronotum length) and day mated (1–41) as covariates revealed a highly significant effect: the number of eggs produced increases with the amount of male-derived nutrition eaten (Table 1). All pairwise comparisons of treatment means were significantly different (Student–Newman–Keuls (SNK) tests, $P < 0.05$). The

Table 2 Effect of number of spermatophylaxes eaten on egg weight

No. of spermatophylaxes eaten	0	1	3	7		
Mean weight of 5 eggs (mg) ($\bar{x}$ + s.e.)	9.7 ± 0.16	10.0 ± 0.18	10.3 ± 0.18	10.6 ± 0.21		
Sample size	14	12	12	13		
Analysis of variance						
	Slope	SS	d.f.	MS	<i>F</i>	<i>P</i>
Treatment		4.44	3	1.48	3.48	0.024
Covariates:						
Day mated	-0.005	0.12	1	0.12	0.27	0.604
Pronotum length	0.22	0.59	1	0.59	1.40	0.245
Explained		5.78	5	1.16	2.72	0.031
Residual		19.2	45	0.426		

consumption of the spermatophore meal in the female's first mating represents an increase in numbers of eggs produced of some 40% (compare treatments 0 and 1, Table 1). The covariates, size and day mated, each had a significant effect (positive and negative, respectively) on egg number (Table 1). A preliminary analysis of covariance demonstrated that the slopes of size and day mated on egg number did not differ between treatments ($P > 0.05$).

Analysis of variance of egg weight (mean weight of groups of five eggs for each female) by treatment with size and day mated controlled revealed a significant effect of treatment; egg weight increases with the amount of male-derived nutrition eaten (Table 2). Only one pairwise comparison (0 and 7) showed a significant difference (SNK test, $P < 0.05$). Neither size nor day mated had a significant effect on egg weight (Table 2). The slopes of these two covariates on egg weight did not differ between treatments (analysis of covariance, $P > 0.05$).

Thus, an increase in the number of spermatophores eaten not only enhances female fecundity but also increases the size of the eggs she produces. Larger egg size in insects is known to correlate with several components of offspring fitness¹³. Although an increase in either egg size or number *per se* may, in certain circumstances, not represent an increase in reproductive success^{13,14}, a concomitant increase in both probably represents increased fitness of females.

Investment in the spermatophore can be regarded as 'parental'¹⁵ as the investment not only enhances female fecundity but is also a cost to the male^{1,15}. A spermatophore represents a weight loss of up to 40% for the male bushcricket⁷; in *Orchelimum delicatum* there is a reduction in the size of the spermatophore produced in a second mating 24 h after the first⁷. A cost would be reflected in a decreased ability to invest in further offspring¹ if a reduction in this food donation to the female reduced the numbers and size of those eggs subsequently fertilized by the male.

The advantage to females of obtaining additional spermatophore nutrients provides an adaptive basis for female discrimination of males observed in some species; female *R. verticalis* prevent insemination when no spermatophylax is received from the male⁵ and female *Conocephalus nigropleurum* prefer to mate with males able to supply larger spermatophores⁶. Thus, both selection on males to produce fitter offspring and intersexual selection (female choice) may have contributed to the evolution of one of the most elaborate sperm packages known.

I thank W. Bailey, R. Black, C. Codd, I. Dadour, D. Roberts, R. Thornhill for comments on the paper, J. Pryce for help in the laboratory, and R. Black for statistical advice. The work was supported by ARGS (Australia) grant D1-77/15071.

Received 24 May; accepted 7 November 1983.

1. Trivers, R. L. in *Sexual Selection and the Descent of Man, 1871–1971* (ed. Campbell, B.) 136–179 (Aldine, Chicago, 1971).
2. Thornhill, R. *Am. Nat.* **110**, 153–163 (1976).
3. Bowen, B., Codd, C. & Gwynne, D. T. *Aust. J. Zool.* (in the press).
4. Boldyrev, B. T. *Horae ent. Soc. Ross.* **41**, 1–245 (1915).
5. Gwynne, D. T., Bowen, B. & Codd, C. *Aust. J. Zool.* (in the press).
6. Gwynne, D. T. *Anim. Behav.* **30**, 734–738 (1982).

7. Gwynne, D. T. in *Orthopteran Mating Systems: Sexual Competition in a Diverse Group of Insects* (eds Gwynne, D. T. & Morris, G. K.) 337–366 (Westview, Boulder, 1983).
8. Gwynne, D. T. *Science* **213**, 779–780 (1981).
9. Boggs, C. & Gilbert, L. E. *Science* **206**, 83–84 (1979).
10. Mullins, D. E. & Keil, C. B. *Nature* **283**, 567–569 (1980).
11. Schal, C. & Bell, W. J. *Science* **218**, 170–172 (1982).
12. Gangwere, S. K. *Trans. Am. ent. Soc.* **87**, 67–203 (1961).
13. Capinera, J. L. *Am. Nat.* **114**, 350–361 (1979).
14. Smith, C. C. & Fretwell, S. D. *Am. Nat.* **108**, 499–506 (1974).
15. Gwynne, D. T. in *Sperm Competition and the Evolution of Animal Mating Systems* (ed. Smith, R. L.) (Academic, New York, in the press).
16. Gwynne, D. T. *Evolution* (in the press).

Stress-induced alignment of division plane in plant tissues grown *in vitro*

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The architectural nature of cell patterns in plants, which are visible under the light microscope, offers strong intuitive evidence for a structural relationship between mechanical forces acting through the tissue and cell wall orientation at cytokinesis. However, a direct relationship between the two has never been convincingly established. We find that compressive forces at intensities between 500 and 800 mg mm⁻² can induce a conspicuous spatial ordering of division activity when applied to sterile plant tissues during continued growth in culture; this results in cambium-like areas of cell wall co-planarity which are clearly distinguishable from the characteristically disorganized growth of unstressed regions of the explant, and implies the presence of an intracellular structural-mechanical sensor capable of directing the installation of the new cell partition. The experiment reported here, in which compressive forces were applied to plant tissue growing *in vitro*, provides an example of mechanical control of division plane.

Cell division in multicellular plant tissues is characterized by a telophase structure known as the cell plate¹ which is an early stage of partition wall formation. The role of cell plate orientation in the development of form in plants is clear as repeated parallel divisions increase the number of cells in a file, thereby contributing to the directional accumulation of tissue mass. Such file cells also tend to elongate perpendicular to the new cross walls, which means that the overall cross-wall orientation of the

cells comprising an unexpanded organ also predicts the direction of elongation of the organ as a whole.

We propose that the final orientation of the cell plate is adjusted according to extracellular stress-mechanical cues until it assumes an orientation which is architecturally functional, that is, maximally protected from shear². According to our hypothesis, mechanical forces transmitted through the tissue are directly sensed at the cellular level, so that each dividing cell responds to its own stress-mechanical environment with the erection of an appropriately oriented partition wall.

If this is true, then dividing cells subjected to external forces acting in a given direction should respond by orienting new partition walls consistently with respect to the direction of force propagation. This has been shown for whole plants *in vivo*³, but never for isolated tissues growing *in vitro*. Cultured plant tissues subjected to exogenous forces should provide particularly clear-cut support for this hypothesis because: (1) They may be considered to be largely stress-relieved after explanting and transfer to sterile culture, so that any induced order can be unequivocally attributed to the stresses and strains generated by the experimentally applied forces. (2) The physiological environment of growth can be more closely controlled, making it more difficult to attribute induced order to concerted hormonal responses and morphogen gradients which could be organized by a whole plant. (3) Tissues can be chosen which are virtually homogeneous and devoid of structurally specialized cell types which could modify the transmission of forces through the tissue. The freshly explanted pith tissue used here is homogeneous and regular, but not ordered into ranks and files as are the products of an *in vivo* vascular cambium (Fig. 1A). Thus, the experimentally induced co-planarity cannot be ascribed to pre-existing order in the explant.

Fresh pith cores weighing ~25 mg were taken from the second internode of greenhouse-grown *Nicotiana tabacum* L. (Fig. 1A) with a square coring tool, providing a square pith explant 1 mm on a side and of indefinite length. The apical centimetre of this explant was removed and 'planted' in its original orientation in an agar-solidified medium, which was then layered with 50 ml of identically constituted liquid medium. We used a standard defined medium⁴ with added indole 3-acetic acid (IAA; 10 mg l⁻¹) and kinetin (0.4 µg l⁻¹), and incubated the explants at 28 °C.

After a 24-h initial accommodation period to allow for a transient burst of elongation growth, compressive forces were applied to the tissue by means of a pneumatic forcing frame

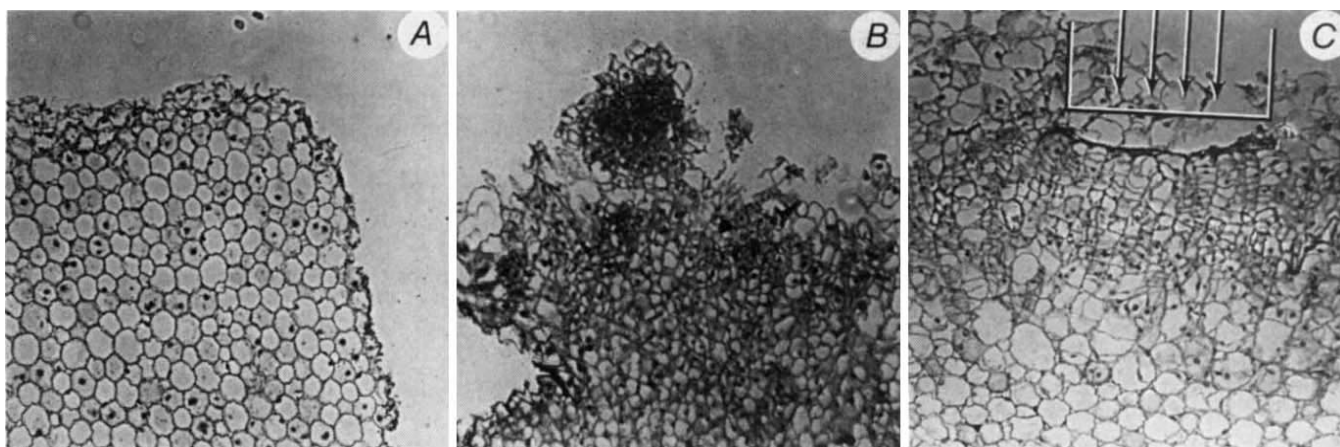
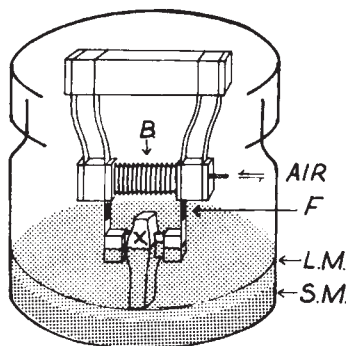


Fig. 1 Transverse sections of cultured and uncultured explants of *Nicotiana* pith. $\times 100$. **A**, The initial condition of the explant before transfer into sterile culture. Note the lack of differentiated structural or vascular elements and of regular, co-oriented partition walls. Interfascicular cambium is absent from this level of the stem. **B** And **C** show aspects of an equivalent explant after 5 days in a standard defined medium. **B** Shows unrestrained callus proliferating from one corner of the explant; note the random proliferation, with little evidence of repeated divisions in any one plane. **C** Shows another area of the same section, growing under continuous compressive stress applied through a narrow Teflon interface (arrows). Note the radially aligned files of cells extending from the original pith core out to the interface surface. These files of cells result from repeated, co-planar divisions of pith-derived cells, constrained by 0.075 bar compressive stress. Parallel development gives the region a cambium-like appearance. Some evidence of xylem differentiation can be seen at a level 6–8 cells internal to the interface. Some loose callus cells have slumped into the interface area during fixation.

Fig. 2 Schematic representation of a pneumatic forcing-frame developed for continuous, long-term compressive loading of tissues in sterile culture. The sterilizable assembly hangs from the lid of a standard culture dish. Bellows B is the actuator, connected to an external peristaltic pump which reversibly meters small amounts of air into the bellows. A continuous matching of the applied force to a preset force set-point is accomplished by an external feedback control circuit (not shown). Forces applied to the tissue are measured by resistive strain gauges at F, which act as force transducers. The tissue explant is partly embedded in a layer of solidified medium (SM), over which is poured a layer of liquid medium (LM). Partly submerged Teflon interface pieces attached directly to the force transducers apply forces directly to the tissue at the meniscus. Each of the opposed contact surfaces measures 10.0×0.5 mm.



(Fig. 2). In the experiment reported here the frame squeezed opposing vertical faces of the explant.

A rapidly developing callus growth at the air-liquid meniscus soon supported the entire load generated by the forcing frame. At the end of the experiment, forces of 0.75 g were supported by an area of callused explant surface somewhat larger than 1 mm^2 , generating compressive stress intensities of 0.075 bar at the contact surface. After 5 days of active proliferative growth while being subjected to continuous, controlled compressive stress, the explant, now weighing ~ 250 mg, was fixed and sectioned in a plane perpendicular to its long axis.

The effective resolution of force measurement in our system is ± 10 mg. There is also a cumulative error due to instrumental drift, which in the experiment presented here resulted in the gradual increase in applied force from an initial value of 500 mg to a final value of 750 mg after 5 days. These instrumental difficulties, together with the fact that not all explants are completely alike or grow equally rapidly and uniformly, have prevented us from obtaining a true picture of the repeatability of our result. Nevertheless, in all explants where compressive stresses between 500 and 800 mg mm^{-2} were applied to actively dividing cells, co-planar divisions were evident, although not always extending uniformly across the interface surface. Further development of our instrument and methods should allow us to routinely effect cell alignments like those illustrated here.

Fig. 1C shows that co-planar divisions immediately internal to the interface surface accumulated to a depth of 10 ranks of cells, giving the load-bearing region of the explant an appearance similar to that produced by cambial activity. By comparison, unloaded regions of the same section show the largely disorganized callus growth behaviour typical of this tissue in this medium (Fig. 1B).

The proliferation of the explanted tissue at the meniscus is presumably due to an interplay of factors from the medium and from the air which provide an optimum of nutrition, gas exchange and surface effects. However, the proliferation encircles the explant at the meniscus, whereas the region of co-planar divisions is restricted to those parts of the proliferation supporting the directional compressive forces generated by the forcing frame. This implies that mechanical stimulation is the principal effector responsible for the co-planar orientation of newly installed cell partitions in this experimental system.

Although many plant tissues, when explanted into sterile culture, can be induced to undergo a normal morphogenesis, most will revert temporarily to a disorganized growth form termed callus. Tobacco pith is one of these. We propose that this behaviour is due to the loss of the *in vivo* mechanical environment of growth, which no amount of *in vitro* nutritional or hormonal manipulation can restore. The experiment reported

here is a direct attempt to re-establish this lost mechanical environment. The distribution, coherence and regularity of divisions in the example presented here are difficult to explain by a diffusively maintained prepattern or positional signal⁵ which would have to be focused through the tissue and interpreted by the individual cells in such a way as to orient new partition walls with strict regard to external landmarks.

A mechanism responding directly to stress-mechanical stimuli can refer to the intracellular physical effects of the stresses and strains propagating through the tissue for local spatial cues, and requires only that each cell is provided with an intracellular sensor which can react in predictable ways to the slight cellular dimension changes resulting from the directional transmission of force through the tissue². The regularity of the response then becomes simply a function of the predictability of stress propagation through the tissue, which in turn depends on the quality of the mechanical coupling of the tissue provided by the shared cell walls. The need to assign unique positional values to each cell⁵ disappears, as the stress-mechanical environment of each cell is necessarily location-specific.

Throughout the plant kingdom, growth and development provide repeated examples of highly ordered and conserved division sequences which offer *prima facie* evidence of structural adaptation at the tissue level. Although this is one of the oldest and most recurrent themes of plant science^{6,7}, a solid experimental and theoretical basis for understanding the structural sensitivities of individual plant cells is absent from the literature.

We hope that the direct manipulation of the plane of division in actively growing plant tissues *in vitro* will lead to a more precise understanding of developmental events in plants by providing a tool for the dissection of the constraints which govern cell wall patterns.

Received 25 August; accepted 2 November 1983.

1. Hepler, P. K. *Protoplasma* **111**, 121-133 (1982).
2. Lintilhac, P. M. in *Positional Controls in Plant Development* (ed. Barlow, P.) (Cambridge University Press, in the press).
3. Lintilhac, P. M. & Vesecky, T. *Am. J. Bot.* **67**, 1477-1483 (1980).
4. Murashige, T. & Skoog, F. *Physiologia Pl.* **15**, 473-497 (1962).
5. Holder, N. J. *theor. Biol.* **77**, 195-212 (1979).
6. Kny, L. *Jb. wiss. Bot.* **37**, 55-98 (1902).
7. Sinnott, E. W. *Plant Morphogenesis*, 43-54 (McGraw-Hill, New York, 1960).

Prevention of gastrulation but not neurulation by antibodies to fibronectin in amphibian embryos

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Gastrulation and formation of the neural plate are major steps in early vertebrate embryogenesis. Although morphogenetic movements leading to the formation of the primary germ layers have been extensively described¹⁻³, the mechanisms governing migration of mesodermal cells and their interactions with ectoderm remain ill-defined. A large body of evidence indicates that fibronectin (FN), a high molecular weight cell-surface-associated glycoprotein, promotes cell adhesion and cell migration throughout embryogenesis⁴⁻⁶. FN has been detected at an early blastula stage in *Pleurodeles waltlii*⁷. We now show that FN is a component of a dense fibrillar matrix underlying the blastocoel roof; in contrast, the exterior surface of the embryo

is devoid of FN. Microsurgical inversion of part of the blastocoel roof does not prevent mesodermal cell migration except at the site of inversion where no FN matrix is available. Perturbation experiments using antibodies to FN demonstrate that the invagination of presumptive mesodermal cells does not occur when the monovalent antibodies are injected before or at the onset of gastrulation; on the other hand, the formation of a neural plate is not prevented when late gastrula stage embryos are treated with antibodies to FN. We conclude that the presence of FN is required for cell migration during gastrulation.

Antibodies directed against chick FN have been previously used to demonstrate the presence of FN on the inner surface of the ectoderm of gastrulating *Pleurodeles* embryos⁷. Specific antibodies to *Ambystoma mexicanum* plasma FN were prepared (Fig. 1) to improve the immunofluorescence staining. In whole-mount ectodermal explants, we could visualize a dense fibrillar FN-rich extracellular matrix on the inner surface of the blastocoel roof (Fig. 2a–c). In contrast, no FN could be detected (Fig. 2c) on the outer surface of the embryo.

To probe for a potential role of the extracellular matrix in gastrulation, part of the roof of the blastocoel was grafted inside-out near the site of invagination (Fig. 2d). In these embryos pioneer migrating mesodermal cells avoided the grafted region. At a later stage, the confluent mesodermal layer that formed uniformly beneath the ectoderm was found to overlap this region without adhering to it (Fig. 2e).

Injection of monovalent antibodies to FN into the blastocoel cavity just before gastrulation prevented the invagination of mesodermal cells. The gross appearance of the embryo (Fig. 2f) was strikingly different from that of the control (Fig. 2g). The blastocoel roof gave rise to a highly convoluted structure, possibly because it could not undergo normal epiboly. The lower surface corresponded to the non-invaginated presumptive mesodermal and endodermal cells. Only a very few pioneer migrating cells were found on the inner surface, which remained mostly naked (not shown).

Antibodies to FN were found to bind to the FN fibres at the surface of the blastocoel for at least 12 h after injection (not shown). Table 1 summarizes the results obtained at different stages before and during gastrulation. When monovalent antibodies to FN were injected in the blastocoel cavity at the blastula or early gastrula stages, a clear-cut inhibition of gastrulation was observed within 6 h. This phenomenon lasted for 24 h and led to abnormal development of the embryo as shown in Fig. 2f. Interestingly, when gastrulation was almost completed, injection of antibodies to FN did not affect neurulation. Some embryos did not survive 24 h; this was not due to a nonspecific toxic effect of the antibodies, since no cytolysis was observed

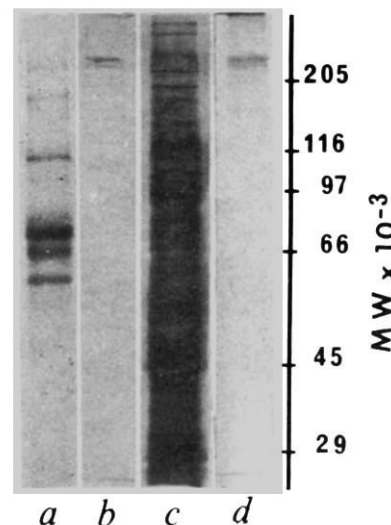


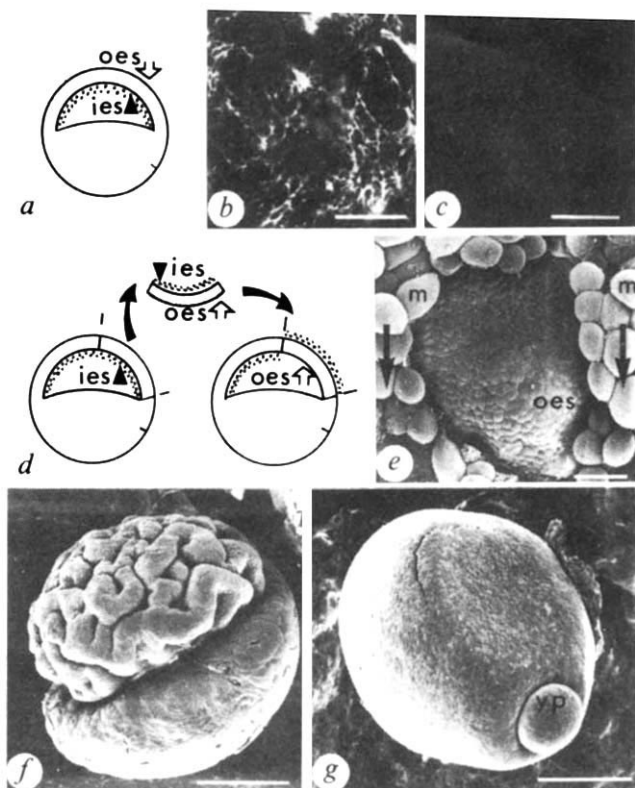
Fig. 1 Specificity of antibodies directed against *Ambystoma mexicanum* FN. *Ambystoma mexicanum* plasma FN was purified by affinity chromatography on a gelatin Sepharose column²⁰ and high titre (1:6,000 dilution for immunofluorescence labelling) antibodies were raised in rabbits. IgG and Fab monovalent fragments were prepared according to Brackenbury *et al.*²¹, 10% polyacrylamide gel electrophoresis²² and immunoblotting²³. *a, b*, Plasma from adult *Ambystoma mexicanum*. *a*, Several major bands are detectable after Coomassie blue staining. *b*, Only one close doublet of molecular weight (MW) 220,000 was revealed after transfer to nitrocellulose sheets, followed by incubations with rabbit IgG anti-FN (2 µg ml⁻¹) and Protein A coupled to peroxidase (5 µg ml⁻¹) and revealed with diaminobenzidine in the presence of H₂O₂ (standard MWs correspond to myosin (205,000), galactosidase (116,000), phosphorylase *b* (97,000), bovine serum albumin (66,000), ovalbumin (45,000) and carbonic anhydrase (29,000)). *c, d*, Electrophoresis of *Pleurodeles waltlii* mid-gastrulae embryos solubilized in SDS sample buffer²². *c*, Coomassie blue staining shows more than 50 bands. *d*, Only one doublet of MW 220,000 reacted with anti-FN antibodies. The protein recognized both in the plasma and in the protein mixture extracted from embryos co-migrated with purified plasma FN. These antibodies were considered to be monospecific and were not used routinely as affinity purified because their titre decreased considerably with this procedure which prevented inhibition experiments.

Table 1 Effects of monovalent fibronectin antibodies on the gastrulation of *Pleurodeles waltlii* embryos

Stages		6 h		24 h	
		Normal (%)	Blocked (%)	Normal (%)	Blocked (%)
Fab anti FN	Blastula (20)	20	80	20	80 (25)
	Early gastrula (16)	—	100	—	100 (18)
	Late gastrula (15)	80	20	80	20
Preimmune Fab'	Blastula (20)	100	—	100	—
	Early gastrula (25)	100	—	100	—
Fab'anti FN + FN	Blastula (25)	90	10	90	10
	Early gastrula (30)	93	7	93	7
BSA	Blastula (30)	100	—	100	—
	Early gastrula (15)	100	—	100	—
Steinberg solution	Blastula (12)	100	—	100	—
	Early gastrula (15)	100	—	100	—

Embryos were injected at three stages of development as described in the legend to Fig. 2 (number of treated embryos for each stage is given in parentheses); in all cases, monovalent antibodies and bovine serum albumin (BSA) were used at 20 mg ml⁻¹; the final concentration (4–10 mg ml⁻¹) in the blastocoel cavity cannot be estimated rigorously. With antibodies to fibronectin, the blastocoel roof of most embryos treated before or at an early stage of gastrulation developed into convoluted structures as in Fig. 2f. Partial necrosis was observed after 24 h in some embryos (percent indicated in parentheses) in which mesodermal cell migration did not occur.

Fig. 2 Distribution and role of FN in *Pleurodeles waltlii* gastrulation. **a–c**, Immunofluorescence labelling for FN: 4% formaldehyde-fixed or living ectoderm explants were incubated in anti-FN IgG ($10 \mu\text{g ml}^{-1}$) for 1 h followed by fluorescein isothiocyanate FITC-conjugated sheep anti-rabbit antibodies diluted 1:100 in 0.5% bovine serum albumin. Explants were whole-mounted in 90% glycerol, examined with epi-illumination through a Leitz Dialux 20 microscope and photographed with Kodak Tri-X films. **a**, Schematic representation of an early gastrula inner (filled triangle) and outer (open arrow) surface of the blastocoel roof. **b**, Staining for FN on the inner surface of the blastocoel roof. An extensive FN-rich fibrillar matrix covers the inner surface of the ectoderm layer. **c**, Unlike the inner surface, the outer surface of the blastocoel shows no fluorescence for FN. **d, e**, Inverted explants were grafted to determine whether the FN-rich extracellular matrix mediates the migration of mesodermal cells. **d**, Schematic representation of the grafting procedure. An inverted ectodermal explant was grafted on the future dorsal side of the embryo above the blastoporal lip. The position of invaginated mesodermal cells was checked after gastrulation had progressed. **e**, Scanning electron micrographs of the blastocoel roof in a gastrula bisected 3 h after grafting. The mesodermal cells attach to the inner surface of ectodermal cells but do not adhere to the outer ectodermal surface of the graft. The overlapping cohesive sheet of mesodermal cells that follows the pioneer cells has been removed. It is clear that adhesion has not taken place between the migrating mesodermal cells and the outer surface of the ectodermal explant (arrows, direction of migration). **f, g**, Injection of monovalent antibodies to FN into the blastocoel. 200 nl of a 20 mg ml^{-1} solution of Fab anti-FN were injected into *Pleurodeles waltlii* blastulae or gastrulae. Similar amounts of preimmune Fab' or bovine serum albumin were injected as controls. Scanning electron micrographs were made 24 h after injection of blastulae. **f**, Gastrulation is almost completely inhibited when antibodies to FN are used; the blastocoel roof becomes folded extensively into disorganized convolutions. In contrast, the inner surface of the ectoderm remains smooth (not shown). The part of the embryo which should have invaginated is found as a cap. A very limited invagination can be observed at the boundary between the cap and the superficial ectoderm. This limited invagination can be visualized after removal of the ectoderm; however, mesodermal cells did not develop many filopodia and lamellipodia over the surface of the ectoderm and occupy only a very limited area as opposed to their homogeneous distribution over the entire surface of the ectoderm during normal development (not shown). **g**, Embryos injected with monovalent preimmune antibodies develop normally with only a small yolk plug (yp) at the site of invagination. For scanning electron microscopy, embryos or explants were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4–7.6, at room temperature and postfixed in 1% OsO_4 in the same buffer. Samples were dehydrated through a graded ethanol series, critical point dried, coated with gold, examined with a Jeol JSM-35 scanning electron microscope at 25 kV and photographed with Ilford FP4 film. Scale bars: **b, c**, $20 \mu\text{m}$; **e**, $100 \mu\text{m}$; **f, g**, $320 \mu\text{m}$. ies, Inner ectodermal surface; m, mesodermal cell; oes, outer ectodermal surface; yp, yolk plug.



24 h after injection of the same amount at the late gastrula stage. In control experiments, all the embryos reached the neurula stage when injected with preimmune monovalent antibodies, with Steinberg solution or with bovine serum albumin; in addition embryos cultivated in the presence of antibodies to FN develop normally (not shown). Most importantly, when antibodies to FN were preincubated with *Ambystoma mexicanum* FN, gastrulation proceeded normally (Table 1).

In gastrulating amphibian embryos, the presence of an extracellular matrix was suggested only recently^{8,9}. Its early appearance was documented *in vivo* using scanning electron microscopy and a possible function as a guiding structure for migrating mesodermal cells was suggested¹⁰. Migration of amphibian mesodermal cells was also observed *in vitro* on the extracellular matrix transferred from ectoderm explants¹¹. However, although it has been reported that FN mediates adhesion of mesodermal cells *in vitro*, it has not been shown so far that these cells utilize FN *in vivo* as a substrate for migration¹². We have previously shown that FN is present at the blastula stage⁷ and we establish here that it is a component of the fibrillar extracellular matrix within which mesodermal cells migrate.

The presence of the extracellular matrix could be required for attachment in grafting experiments (Fig. 2d); however, this experiment could merely indicate an ability of mesodermal cells to adhere only to basal surfaces. The importance of FN was clearly assessed by using specific monovalent antibodies to FN. Nevertheless we cannot exclude that other ECM components may also be essential for attachment and migration of mesodermal cells. It should be stressed that in itself FN does not trigger the process of invagination since it is already incorporated

into the extracellular matrix prior to gastrulation. The early deposition of FN was also observed in chick embryos before gastrulation^{13–15} and before neural crest cell emigration¹⁶. It is likely that mesodermal cells have an intrinsic ability to respond to the FN-rich matrix with which they interact directly. However, it is not clear whether the matrix fibrils are oriented in the direction of movement. Rather, confluency at all stages of migration may allow the cells to migrate unidirectionally as a result of population pressure, a mechanism also suggested by Kubota and Durston¹⁷ for amphibians and by Rovasio *et al.*⁵ for avian neural crest cells.

Once migration of mesodermal cells was almost complete, antibodies to FN did not perturb the formation of a neural plate. However, we cannot exclude that antibodies to FN introduced at the late gastrula stage had no effect since interactions between mesodermal and ectodermal cells leading to neurulation may not last more than several hours¹⁸. Nevertheless, the presence of a FN-rich extracellular matrix does not seem to be required, as a direct association of mesodermal cells with the outer surface of the ectoderm leads to neurulation¹⁹.

This work was supported by grants from the Ministère de l'Industrie et de la Recherche (82 E 1139, 81-E1082) and the Ministère de l'Éducation Nationale. Professors M. Jacobson and L. Saxen and Drs K. Yamada and J. Smith made a critical reading of the manuscript. We thank L. Addade and P. Groue for technical assistance.

Received 17 June; accepted 2 November 1983.

- Holtfreter, J. *J. exp. Zool.* **94**, 261–318 (1943).
- Holtfreter, J. *J. exp. Zool.* **95**, 171–212 (1944).
- Vakaet, L. *Archs Biol.* **81**, 387–426 (1970).
- Hynes, R. O. & Yamada, K. M. *J. Cell Biol.* **95**, 369–377 (1982).

5. Rovasio, R. A., Delougee, A., Yamada, K. M., Timpl, R. & Thiery, J. P. *J. Cell Biol.* **96**, 462-473 (1983).
6. Wylie, C. C. & Heasman, J. *Phil. Trans. R. Soc. B* **299**, 177-183 (1982).
7. Boucaut, J. C. & Darribere, T. *Cell Tissue Res.* **234**, 135-145 (1983).
8. Tarin, D., *J. Embryol. exp. Morph.* **26**, 543-570 (1971).
9. Keller, R. E. & Schoenwolf, G. C. *Wilhelm Roux' Arch. dev. Biol.* **182**, 165-186 (1977).
10. Nakatsuji, N. & Johnson, K. E. *J. Cell Sci.* **59**, 61-70 (1983).
11. Nakatsuji, N. & Johnson, K. E. *J. Cell Sci.* **59**, 43-60 (1983).
12. Nakatsuji, N. & Johnson, K. E. *Cell Motil.* **2**, 149-161 (1982).
13. Critchley, D. R., England, M. A., Wakely, J. & Hynes, R. P. *Nature* **280**, 498-500 (1979).
14. Sanders, E. J. *J. Embryol. exp. Morph.* **71**, 155-170 (1982).
15. Duband, J. L. & Thiery, J. P. *Dev. Biol.* **94**, 337-350 (1982).
16. Newgreen, D. & Thiery, J. P. *Cell Tissue Res.* **211**, 269-291 (1980).
17. Kubota, H. Y. & Durston, A. J. *J. Embryol. exp. Morph.* **44**, 71-80 (1980).
18. Duprat, A. M., Gualandris, L. & Rouge, P., *J. Embryol. exp. Morph.* **70**, 171-187 (1982).
19. Gualandris, L., Rouge, P. & Duprat, A. M., *J. Embryol. exp. Morph.* (in the press).
20. Engvall, E. & Ruoslahti, E. *Int. J. Cancer*, **20**, 1-5 (1977).
21. Brackenbury, R., Thiery, J. P., Rutishauser, U. & Edelman, G. M. *J. biol. Chem.* **252**, 6835-6840 (1977).
22. Laemmli, U. K. *Nature* **227**, 680-689 (1970).
23. Towbin, M., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350-4354 (1979).

Cultivation of the liver forms of *Plasmodium vivax* in human hepatocytes

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The blood schizogonic cycle of human malaria parasites has thus far been the most exhaustively studied phase of parasite development. However, before entering red blood cells (RBCs), the parasite undergoes its first multiplication not in blood, but in hepatic cells. These hepatic stages were the last to be discovered¹ and only a few studies have been performed in humans and other primates¹⁻⁷. Despite recent advances⁸⁻¹¹, *in vivo* studies have limitations and other approaches such as cultures of these liver forms may be necessary to investigate their chemosensitivity and their biochemical or immunological properties. Recently, sporozoites of species of rodent malaria have been made to infect cultured cell lines¹²⁻¹⁵ or primary hepatocyte cultures¹⁶⁻¹⁸. We report here that the complete cycle of the human malaria parasite *Plasmodium vivax* can be obtained in primary cultures of human hepatocytes up to release of merozoites able to penetrate RBCs.

A new method for culturing metabolically active human hepatocytes using enzymatic perfusion has recently been described^{19,20}. In the present experiment, we modified this method by cannulating and perfusing a small blood vessel of a liver biopsy fragment obtained for diagnostic purposes. Preparation of hepatocyte monolayers and culture conditions are the same as previously described using rodent liver cells¹⁷ except that the hepatocytes were seeded at a concentration of 5×10^5 cells per 35-mm Falcon Petri dish in 1.5 ml supplemented minimal essential medium. Such hepatocyte monolayers can easily survive for 1-2 weeks in conventional culture conditions or for several weeks with maintenance of high levels of specific functions when co-cultured with a rat liver epithelial cell line²⁰ (Fig. 1). In this experiment, since a liver biopsy fragment was obtained 2 days before availability of sporozoites, only four out of eight hepatocyte cultures were set up as co-cultures with epithelial cells (added at a concentration of 5×10^5 per dish) while the remaining four were set up as monocultures.

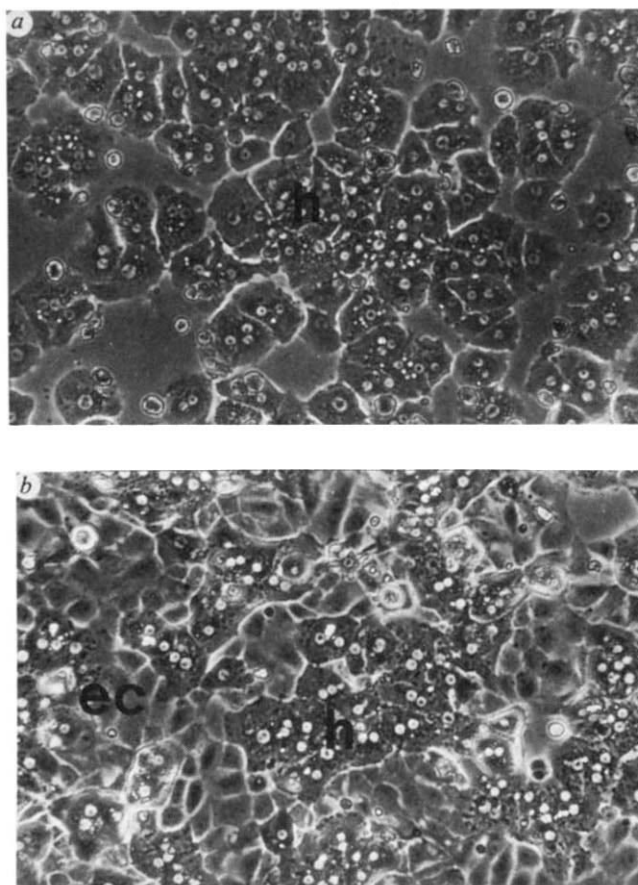


Fig. 1 a, Hepatocytes in monoculture (h). Note the flattened appearance of these cells. In monoculture, the hepatocytes will only remain fully functional for a short period (less than 2 weeks). b, Hepatocytes co-cultured with liver epithelial cells (ec). Hepatocytes appear more rounded and organized into cord-like groups. In these conditions the hepatocytes remain functional for up to 2 months. ($\times 200$.) Phase-contrast micrograph.

Sporozoites of *P. vivax* were obtained from *Anopheles stephensi* mosquitoes infected by membrane feeding with 10 ml of citrated blood containing gametocytes ($5 \times 10^7 \text{ ml}^{-1}$) collected from a patient who had been infected in India. Examination of mosquito midguts on day 11 following the blood meal showed that 15% of them were infected. On day 16, the mosquito salivary glands were aseptically dissected in culture medium. The culture supernatant from each Petri dish was decanted and groups of 10 pairs of salivary glands in 80 μl medium were added to each dish. The preparations were incubated for 45 min at 37 °C, with 5% CO_2 and 95% air after which 0.5 ml of medium was added, followed by an additional 1 ml 5 h later. Subsequently, the medium was changed daily. In one culture fixed on day 5 following sporozoite inoculation and stained with Giemsa, an exo-erythrocytic schizont of small size (10 μm) was identified. In two of three monocultures fixed on day 7 of culture, intracellular schizonts of various sizes were seen (Fig. 2a-d), but only a few were observed in the third culture. The larger ones measured 30 μm and contained up to 200-300 nuclei. Smaller schizonts ranged from 10 to 25 μm . The smallest was 5 μm and contained five nuclei. They were generally located close to the nucleus of the host cell which appeared unaltered by the presence of the schizont except for a condensed area surrounding a clear space around the schizont. Schizonts could be seen over the entire area of the culture but were more numerous in the vicinity of the introduced salivary glands. The number of schizonts seen was relatively high (about 30 in each of two dishes).

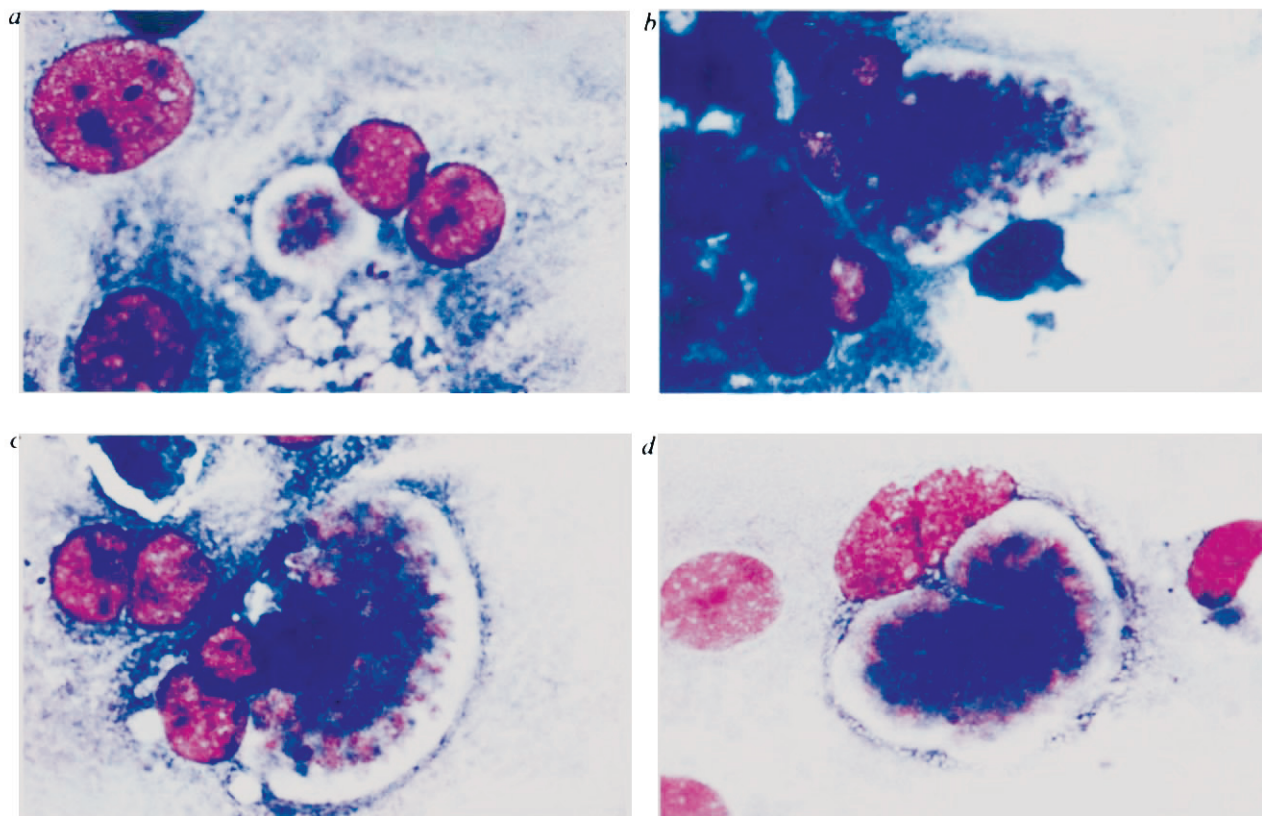


Fig. 2 Seven-day exo-erythrocytic parasites of *P. vivax* in cultural hepatocytes ($\times 1,300$). *a*, Early schizont with only a small number of nuclei. *b*, Maturing schizont with a greater number of nuclei. *c*, *d*, Late schizont undergoing segmentation. Giemsa staining.

To establish unequivocally whether the hepatic schizonts could achieve full maturity *in vitro*, we added RBCs to two hepatocyte co-culture dishes on day 10. Because *P. vivax* preferentially penetrates young RBCs, blood collected from a patient with a 30% reticulocytosis was used. Following two washes in culture medium, this blood was added at a concentration of 10^8 blood cells per culture dish. Thin blood films were made on days 12 and 14. Numerous intra-erythrocytic *P. vivax* parasites were identified on both preparations after staining with Giemsa. Most of the intra-erythrocytic forms were rings, although some were trophozoites containing malarial pigment, and one schizont with eight nuclei was seen. Schüffner's dots were present in many of the infected cells. In the hepatocytes of this latter culture dish fixed on day 14 no intra-hepatocytic parasite could be unequivocally identified.

No uninucleated body of recognizable parasite origin was seen in any of the dishes fixed on days 5, 7 and 14. However, we emphasized that the uninucleated liver forms of *P. vivax* known as hypnozoites, which have been described recently in the liver of infected chimpanzees⁸, might be difficult to find on Giemsa-stained preparations because of their size. Unfortunately the small number of cultures infected in these experiments did not allow us to perform other more specific staining procedures, for example, using labelled antibodies.

It was further shown that the cultured hepatocytes had retained their differentiated functions throughout the experimental period by measuring their ability to produce albumin. Human albumin levels in culture supernatant, as measured by an immunonephelometric method, varied from 2.7 to 4 $\mu\text{g ml}^{-1}$ from day 1 to day 11.

These results demonstrate that the full pre-erythrocytic cycle of *P. vivax* can be completed *in vitro*, using the methods described. It is now important to determine, first, if a human cell is required and, second, if an hepatocyte must be fully differentiated and functional to support penetration of sporozoites and complete exo-erythrocytic development up to

the point of infective merozoite release. Doby and Barker²¹ observed a small number of intra-cytoplasmic bodies (11–21 μm) following inoculation of *P. vivax* sporozoites into a human liver cell culture which had been maintained *in vitro* for at least 1 month before the addition of the sporozoites. Beaudoin and Hollingdale recently reported that *Plasmodium falciparum* and *P. vivax* sporozoites entered cells of a human lung embryonic line (WI38) as well as a hepatoma line (Hep G₂-A₁₆), but that development was not observed²². It is noteworthy that in these previous studies the cells were never fully functional hepatocytes and in no case was complete exoerythrocytic development observed.

These results suggest that, although human malaria parasites may infect a variety of cell types, only the functionally intact hepatocyte may provide the proper conditions for complete exo-erythrocytic development of *P. vivax*. On the other hand, more extensive experiments performed using species of rodent malaria have clearly shown that the specificity for the host cell may not be exclusive^{15–18}. Furthermore, indications gained from *in vivo* studies suggest that the species specificity of the liver forms of human plasmodium may not be as restrictive as for the erythrocytic stages⁹.

One area of considerable interest associated with the cultivation of exo-erythrocytic stages of *P. vivax* could be to provide a new approach to evaluating the merit of the alternative hypothesis on the nature of the secondary exo-erythrocytic cycle, that is, a dormant uninucleated form called hypnozoite, as against a continuing intrahepatic schizogonic cycle.

As *in vitro* cultivation of the erythrocytic stages of malaria has led to major advances in our knowledge of these stages²³, the *in vitro* culture of the liver stages could also open up a wide range of applications. These are limited at present in part by the supply of sporozoites and of human hepatocytes. It is still necessary, therefore, to try to adapt the system to continuous cell lines. However, even if schizont development in other cell types is shown to be possible, it may well be found ultimately

that a homologous, functional hepatocyte is the cell of choice for certain applications, that is, chemotherapeutic studies which would be best performed using the cell type involved in the metabolism of the drugs being examined.

This work was supported by grants from the French Minister of Research and Industry (82.L.0760 and 82.L.0787) and from WHO/UNDP/World Bank/Special Programme for Research and training in Tropical Diseases. We thank Richard L. Beaudoin, Wallace Peters and Francie Tung for reviewing the manuscript and M. Danis, A. Datry, G. Lecso, and V. Soeun, for their helpful cooperation.

Received 1 August; accepted 28 October 1983.

- Shortt, H. E. & Garnham, P. C. C. *Trans. R. Soc. trop. Med. Hyg.* **41**, 785–795 (1948).
- Shortt, H. E., Fairley, N. H., Covell, G., Shute, P. G. & Garnham, P. C. C. *Trans. R. Soc. trop. Med. Hyg.* **44**, 405–419 (1951).
- Rodhain, J. *Ann. Soc. belge Med. Trop.* **36**, 99–103 (1956).
- Bray, R. S. *Am. J. trop. Med. Hyg.* **6**, 514–520 (1957).
- Bray, R. S. *Am. J. trop. Med. Hyg.* **7**, 20–24 (1958).
- Jeffery, G. M., Wolcott, G. B., Young, M. D. & Williams, D. Jr *Am. J. trop. med. Hyg.* **1**, 917–926 (1952).
- Sodeman, T. M., Contacos, P. G., Smith, C. S., Jumper, J. R. & Collins, W. E. *J. Parasit.* **55**, 682–683 (1969).
- Krotoski, W. A. et al. *Am. J. trop. Med. Hyg.* **31**, 1291–1293 (1982).
- Druihlhe, P., Miltgen, F., Landau, I., Rinjard, J. & Gentilini, M. C. *r. hebdom. Séanc. Acad. Sci., Paris* **294**, 511–513 (1982).
- Boulard, Y., Druihlhe, P., Miltgen, F., Landau, I. & Rinjard, S. *Protistologica* **18**, 253–258 (1982).
- Druihlhe, P., Puebla, R. M., Miltgen, F., Perrin, L. & Gentilini, M. *Clin. exp. Immun.* (submitted).
- Strome, C. P. A., de Santis, P. L. & Beaudoin, R. L. *in Vitro* **15**, 531–536 (1979).
- Sinden, R. E. & Smith, J. *Trans. R. Soc. trop. Med. Hyg.* **74**, 134–136 (1980).
- Hollingdale, M. R., Leef, J. L., McCullough, M. & Beaudoin, R. L. *Science* **213**, 1021–1022 (1981).
- Hollingdale, M. R., Leland, P., Leef, J. L. & Beaudoin, R. L. *J. Parasit.* (in the press).
- Lambiotte, M., Landau, I., Thierry, N. & Miltgen, F. *C. r. hebdom. Séanc. Acad. Sci., Paris* **293**, 431–433 (1981).
- Mazier, D. et al. *C. r. Hebdom. Séanc. Acad. Sci., Paris* **294**, 111, 963–965 (1982).
- Pirson, P. *Trans. R. Soc. trop. Med. Hyg.* **76**, 422 (1982).
- Gugen-Guillouzo, C. et al. *Cell Biol. Int. Rep.* **6**, 625–628 (1982).
- GuGuen-Guillouzo, C. et al. *in Isolation, Characterization and Use of Hepatocytes*, 105–110 (eds Harris, R. A. & Cornell, W.) (Elsevier, Amsterdam, 1983).
- Doby, J. M. & Barker, R. C. *r. Séanc. Soc. Biol. Rennes* **170**, 661–665 (1976).
- Beaudoin, R. L. & Hollingdale, M. R. *in Textbook of Malaria* (eds Wernsdorfer, W. & McGregor, I.) (Churchill Livingstone, Edinburgh, in the press).
- Trager, W. & Jensen, J. B. *Science* **193**, 673–675 (1976).

Endogenous ouabain-like compound increases heart muscle contractility

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Cardiac glycosides such as digoxin or ouabain have long been known to influence the strength of contraction of cardiac muscle^{1–3}. Although the mechanism of action of these compounds remains unknown^{4,5}, all the proposed modes of action are based on initial binding to specific membrane receptors which are part of the (Na⁺ + K⁺)ATPase complex. These receptors, well characterized and defined^{6–8}, suggest the existence of an endogenous substance capable of binding to them, in analogy with endogenous opiates, discovered long after morphine and its receptors⁹. As glycosides affect (Na⁺ + K⁺)ATPase activity, an endogenous substance may be a regulator of this important enzyme. Indeed, the search for endogenous regulators of the (Na⁺ + K⁺)ATPase or ouabain-like compounds (OLC) has recently intensified. These compounds, extracted and partially purified from mammalian brain^{10–14}, heart¹⁵, blood^{16–19} and urine^{20,21}, and from toad skin and plasma^{22,23}, have been shown to inhibit ³H-ouabain binding and (Na⁺ + K⁺)ATPase activity. We report here that in addition to these effects, the OLC, highly purified from toad skin and sheep brain, increases the force of contraction of frog and guinea pig atrium.

The toad skin OLC was extracted from 50 g of back and abdominal toad skin (*Bufo viridis*) into 500 ml methanol. The

methanol was filtered and evaporated and the residue redissolved in 20 ml distilled water. Material which was not dissolved was precipitated by high speed centrifugation (100,000g). The supernatant was separated and lyophilized. The residue was dissolved in a minimal volume of methanol (~1 ml) and 20–100-μl aliquots were injected on HPLC. The chromatographic pattern of the separation is shown in Fig. 1a. The main peak with biological activity (that is, inhibits ³H-ouabain binding and (Na⁺ + K⁺)ATPase activity, see below) was obtained at a retention time of 28 min. This peak was collected and rechromatographed on the same system, and the separation is shown in Fig. 1b. The material from the peak at 28 min (second separation) was collected and used in the physiological experiments.

The sheep brain OLC was extracted from brain tissue by acidified acetone and separated on a Sephadex G-25 column as previously described^{12,13}. The biologically active peak obtained from the Sephadex column was further separated on the HPLC column described above (for the toad skin OLC).

Figure 1c shows that the material separated at a retention time of 28 min from toad skin is capable of inhibiting ³H-ouabain binding to synaptosomes prepared from rat brain. The inhibition is dose dependent with half-maximal inhibition at 100 μg of wet weight of skin per ml. By comparing this activity with ouabain (Fig. 1c), and assuming that ouabain and OLC have the same affinity towards their mutual binding site, the concentration of OLC in the skin was calculated to be approximately 0.6 nmol per g tissue, was used to calculate OLC concentrations in the following experiments. The OLC separated at 28 min also inhibited (Na⁺ + K⁺)ATPase activity in the rat brain microsomal fraction, but had no effect on Mg²⁺-ATPase activity (data not shown). These findings confirm previous reports on the presence of ouabain-like compounds in this tissue^{22,23}. Interestingly, the endogenous OLC compound is capable of inhibiting (Na⁺ + K⁺)ATPase activity only at concentrations 100-fold higher than those required to inhibit ouabain binding.

Tension recordings were done from frog atrial trabeculae, placed in a 2-ml chamber and perfused with a normal Ringer solution (for composition see Fig. 2 legend). One end was attached to an AKERS force transducer for measuring the strength of contraction. Constant stimulation was applied at a rate of 0.5 Hz and twitch magnitude was monitored on a pen recorder and oscilloscope and stored on magnetic tape.

Addition of the endogenous, partially purified OLC from toad skin resulted in a marked increase in the force of contraction. This increase was dose dependent and reversible on washout. A positive inotropic effect was sometimes seen at as low a dose as 5 × 10⁻⁸ M, but more commonly doses of 10⁻⁷ M and higher gave significant positive inotropy. An example is shown in Fig. 2a. This effect was seen in seven out of seven cases. The amount of positive inotropy, however, was quite variable in the individual experiments, as indeed is the case with the cardiac glycosides themselves²⁴. A partial dose-response curve was obtained, limited by the quantity of purified compound. Figure 2b illustrates the larger effect produced by the higher doses. No saturation was observed in this limited range. It should be emphasized that, as mentioned above, the concentrations are all calculated from the binding experiments and the true concentrations may actually be different. In one case (Fig. 2c) a high dose of 6 μM produced toxic effects, similar to those obtained with high doses of ouabain²⁵—an increase in the basal (tonic) tension and a reduction in the phasic (twitch) tension.

Several reports suggest that cardiac glycosides exert their inotropic effect by activating the release of endogenous stores of catecholamines^{25–27}. Since our effect was obtained with incompletely identified compounds, we repeated the experiments in the presence of adrenergic blockers. As both α- and β-receptors have been found in several cardiac preparations, including the frog atrium^{28–30}, we used a combination of phenolamine and propranolol, respectively α- and β-blockers. In 10 out of 14 cases, the OLC caused a marked positive inotropic effect in the presence of concentrations between 10⁻⁶ and 5 × 10⁻⁶ g ml⁻¹ of both blockers (Fig. 2d). As both blockers alone

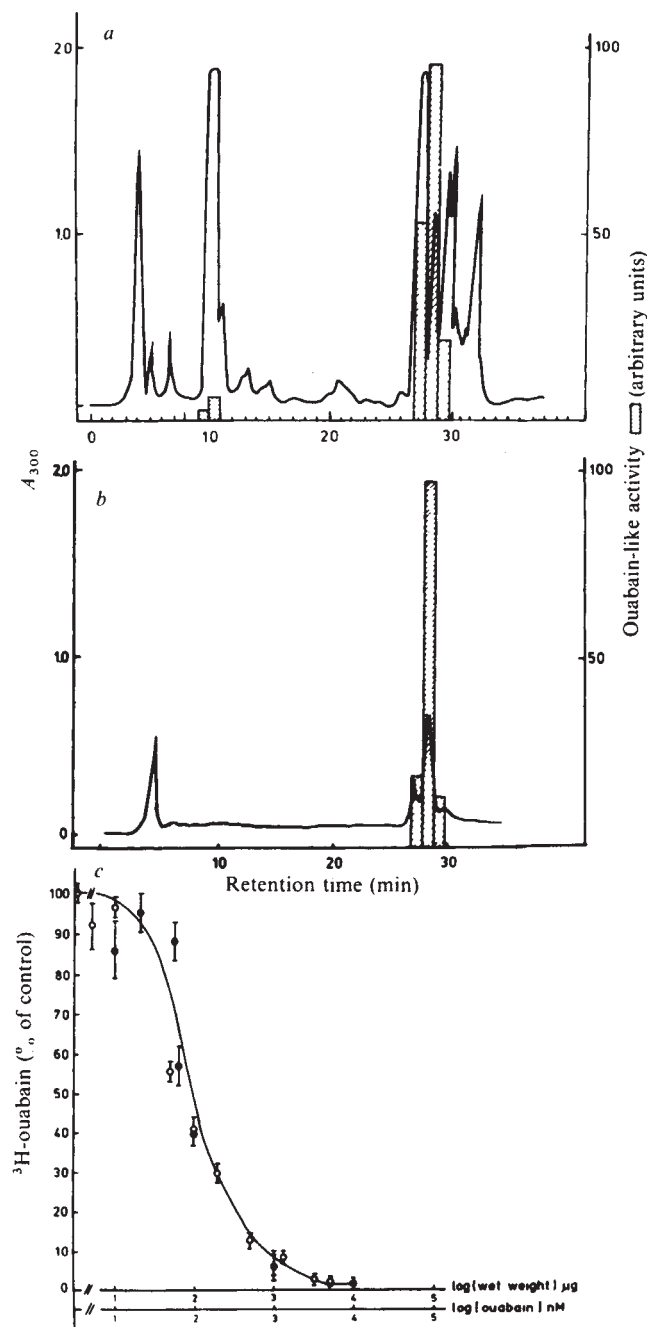


Fig. 1 *a, b*, HPLC of the toad skin OLC and its biological activity. The optical density is plotted (left ordinate) against retention time (abscissa). The right-hand ordinate is the relative biological activity, shown as hatched columns in the graph. This activity was measured by testing the capability of each fraction to inhibit both ^3H -ouabain binding and $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ activity. *c*, Inhibition of ^3H -ouabain binding to rat brain synaptosomes by toad skin extract. The inhibition of binding at different concentrations of ouabain ($\circ$) or OLC ($\bullet$) are shown. The OLC was from samples taken at peak retention time of 28 min.

Methods: *a*, Methanol extract of toad skin (*Bufo viridis*) corresponding to 50 g of skin was dissolved in 1 ml methanol. A Tracor 985 HPLC instrument was used. Aliquots of 20–100 μl were applied to a Alltech amino column (4.6×250 mm, 10 μm particle size), equilibrated with 90% acetonitrile. The column was eluted with linear 90–70% acetonitrile gradient in 40 min, at a flow rate of 1 ml min $^{-1}$. The elution of the compounds was monitored at 300 nm, using a Tracor 970 A UV detector. Fractions of 1 ml were collected, evaporated and the residue was dissolved in 1 ml distilled water. 50- μl samples from this solution were used for measurements of biological activity (*c*). *b*, Fractions (1 ml) with a retention time of 28 min collected from the chromatography in *a* were evaporated to dryness and dissolved in 0.5 ml methanol. Aliquots of 20 μl were applied to the same HPLC system described above. The fractions with a retention time of 28 min were collected, evaporated to dryness and stored at -20°C for 1–6 weeks. These samples were used in the physiological experiments described in Figs 2 and 3. *c*, The methods for synaptosome preparation have been described elsewhere¹². ^3H -ouabain binding was determined by incubating about 0.2 mg synaptosomal protein in 0.5 ml medium, containing (in mM): Tris-HCl buffer (pH 7.4) 50, EDTA 0.5, NaCl 80, MgSO_4 4, ATP 2 (Tris, vanadium-free), 32 nM ^3H -ouabain (NEN, 19.5 Ci mmol $^{-1}$), with or without toad skin extract. Following incubation for 60 min at 37°C , at which time a binding equilibrium is achieved, the reactions were terminated by the addition of 3 ml of cold 50 mM Tris-HCl (pH 7.4), and filtration over Whatman GF/B filters. Filters were washed twice with 3 ml of the same Tris buffer, dried and assayed for radioactivity in a Packard liquid scintillation counter at counting efficiency of ~35%. Specific binding was calculated by subtracting the binding observed in the presence of 100 μM unlabelled ouabain. Specific binding represented 93% of the total binding in control conditions. The control binding in the absence of extract or unlabelled ouabain was 12.5 pmol ^3H -ouabain per mg protein, and was taken as 100%.

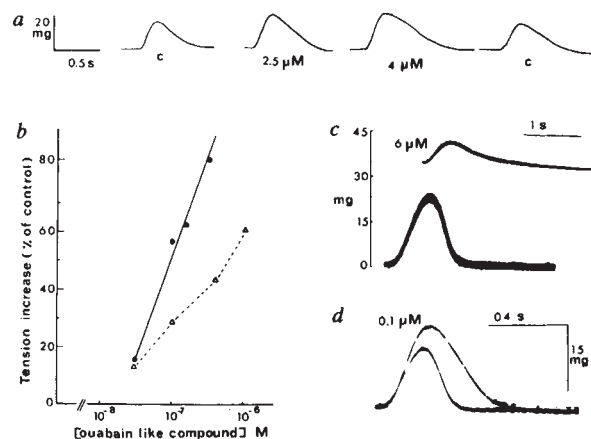


Fig. 2 *a*, The positive inotropic effect of the toad skin OLC on frog atrium. Pen recorder tracings, obtained from frog atrial trabeculae (4–6 mm long, 200–400 μm diameter), during constant 0.5-Hz stimulation. From left to right are the responses in control (c) Ringer, following addition of 2.5 μM OLC, 4 μM OLC and following 20 min washout (c). Control Ringer solution contained (in mM): NaCl 90, NaHCO_3 20, KCl 2, CaCl_2 1.1, MgCl_2 1.0, Na_2HPO_4 0.4, glucose 5.5, bubbled with a 95–5% O_2 – CO_2 mixture. The pH was 7.6–7.7 and the temperature 21 – 24°C . Longer washouts gave more complete reversal of the positive inotropic effect. *b*, A partial dose-response curve. In two cases, four concentrations of OLC were tested. The plot gives the tension increase versus dose (see text for discussion of concentrations of OLC and their estimation). The doses were given cumulatively, with steady state being attained at each dose, before addition of the higher dose. In other experiments two or three doses were used, giving larger effects at higher doses. *c*, A toxic effect of OLC. At a higher concentration (6 μM in this single case) toxic manifestations developed, similar to those obtained with high ouabain levels. The plot shows the elevation in baseline tension as well as the reduced twitch, in the presence of 6 μM OLC, as compared with the control twitch. *d*, The effect of OLC in the presence of α - and β -blockers. Two superimposed oscilloscope records of tension: in a 'control' Ringer solution, containing also 5×10^{-6} g ml $^{-1}$ phentolamine and propranolol (smaller twitch), and after the addition of 0.1 μM OLC (larger twitch).



Fig. 3 The positive inotropic effect of sheep brain OLC on sheep heart. OLC was extracted from seven sheep brains by acidified acetone, and partially separated by Sephadex G-25 chromatography, as previously described^{12,13}. The active material obtained from this separation was further purified using the HPLC system described in Fig. 1a legend. The OLC concentration was estimated by comparing its ability to displace ³H-ouabain binding to rat synaptosomal membranes. The upper tracings show the superposition of tension traces obtained from a sheep ventricular trabeculum. The smaller tension is in a control solution (containing, in mM: NaCl 140, KCl 5.4, NaHCO₃ 12, CaCl₂ 2, MgCl₂ 1, Na₂HPO₄ 0.4, glucose 5, continuously bubbled with 95–5% O₂–CO₂, with a pH of 7.4 at 36 °C). The larger tension response was obtained following the addition of 5×10^{-8} M sheep brain OLC. The positive inotropy was fully reversed on washout. The vertical bar equals 20 mg, as well as 40 mV. The lower traces show the superimposed action potentials, recorded with an intracellular, 3 M KCl microelectrode. There was no significant change in resting potential or in the action potential configuration, aside from a possible small elevation in the plateau phase.

have negative inotropic effects, the two cases without effect and the two in which a negative inotropic effect was produced were attributed to persistent, delayed blocker action.

We next tested this compound on a mammalian preparation to investigate a possible more general applicability. In three experiments on atrial trabeculae from guinea pig hearts, the OLC gave a large positive inotropic response (data not shown). In one case, the same effect was produced in the presence of 5×10^{-6} g ml⁻¹ α - and β -blockers.

Brain OLC was previously shown to bind to ouabain receptors and to inhibit (Na⁺ + K⁺)ATPase activity^{10–14}. In a single trial (Fig. 3), it produced a reversible positive inotropic effect on a trabeculum from sheep ventricle. This 22% increase in contractile force was accompanied by very little change in the action potential configuration, as measured by an intracellular microelectrode.

The chemical nature of the OLC is obviously of major importance. Partial characterization using Sephadex columns and sensitivity to proteolytic enzymes (data not shown) reveals that the toad skin and brain OLCs are of low molecular weight (<500) and are not peptides. It is well known that the toad skin contains toxins which are capable of inhibiting (Na⁺ + K⁺)ATPase, the bufodienolide toxins³¹. It is therefore possible that the OLC separated by us are these steroidal toxins. Experimental results, however, do not favour this possibility: (1) The retention time on the HPLC system described above is in the range of 5–10 min for steroidal compounds (such as ouabain itself), which does not correspond to the main peak of activity found (retention time of 28 min). (2) Only ~5% of the biological activity present in the 28-min peak (aqueous solution) partitioned into 10 volumes of chloroform or ether, indicating that the active principle is relatively polar, unlike the bufodienolides. (3) The UV spectrum of bufodienolides shows a peak at 300 nm, while the spectrum of the compound separated by us shows a maximum at 278 nm and a shoulder at 300 nm (data not shown).

In conclusion, we have shown that the endogenous OLCs extracted from toad skin and mammalian brain are capable of increasing the force of cardiac muscle contraction. This raises the possibility that they serve as physiological regulators of cardiac function. Clearly, further investigation is needed.

This study was supported by the Israel National Academy of Sciences and the Israel Ministry of Health.

Received 7 June; accepted 7 September 1983.

1. Cattel, M. & Gold, H. *J. Pharmac. exp. Ther.* **62**, 116–125 (1938).
2. Cotten, M. & Stopp, P. E. *Am. J. Physiol.* **192**, 114–120 (1958).
3. Langer, G. A. & Serena, D. A. *J. molec. cell. Cardiol.* **1**, 65–70 (1970).
4. Lee, K. S. & Klaus, W. *Pharmac. Rev.* **23**, 194–250 (1971).
5. Noble, D. *Cardiovasc. Res.* **14**, 495–514 (1980).
6. Baker, P. F. & Willis, J. P. *J. Physiol., Lond.* **224**, 441–462 (1972).
7. Akera, T. & Brody, T. M. *Pharmac. Rev.* **29**, 187–220 (1978).
8. Wallick, E. T., Lane, L. K. & Schwartz, A. A. *Rev. Physiol.* **41**, 397–411 (1979).
9. Hughes, J. *et al. Nature* **258**, 577–579 (1975).
10. Hauptert, G. T. Jr & Sancho, J. M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4658–4660 (1979).
11. Fishman, M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4661–4663 (1979).
12. Lichtstein, D. & Samuelov, S. *Biochem. biophys. Res. Commun.* **96**, 1518–1523 (1980).
13. Lichtstein, D. & Samuelov, S. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1453–1456 (1982).
14. Schwartz, A. *et al. Ann. N. Y. Acad. Sci.* **402**, 253–271 (1982).
15. De Pover, A., Castaneda-Hernandez, G. O. & Godfraind, T. *Biochem. Pharmac.* **31**, 267–271 (1982).
16. Poston, L. *et al. Br. med. J.* **232**, 847–379 (1981).
17. Hamlyn, J. M. *et al. Nature* **300**, 650–652 (1982).
18. Gruber, K. A., Whiteker, J. M. & Buckalew, V. M. *Nature* **287**, 743–745 (1980).
19. Cloix, J. F., Miller, E. D., Pernollet, M. G., Devynck, M. A. & Meyer, P. C. *r. hebdo. Séanc. Acad. Sci., Paris* **296**, 213–216 (1983).
20. de Wardener, H. E. & Clarkson, E. M. *Clin. Sci.* **63**, 415–420 (1982).
21. Clarkson, E. M., Raw, S. M. & de Wardener, H. E. *Kidney Int.* **16**, 710–421 (1979).
22. Flier, J. S., Maratos-Flier, E., Pallotta, J. A. & McIsaac, D. *Nature* **279**, 341–343 (1979).
23. Flier, J. S., Edwards, M. W., Daly, J. J. & Myers, C. W. *Science* **208**, 503–505 (1980).
24. Hart, G., Noble, D. & Shimoni, Y. *J. Physiol., Lond.* **334**, 103–131 (1982).
25. Deslauriers, Y., Ruiz-Ceretti, E., Schanne, O. F. & Payet, M. D. *Can. J. Physiol. Pharmac.* **60**, 1153–1159 (1982).
26. Tanz, R. *J. Pharmac. exp. Ther.* **144**, 205–213 (1964).
27. Seifen, E. *Br. J. Pharmac.* **51**, 481–490 (1974).
28. Koch-Weser, J. *Circulation Res.* **28**, 109–118 (1971).
29. Niedergerke, R. & Page, S. *Proc. R. Soc. B213*, 325–344 (1981).
30. Hordof, A. J., Rose, E., Danilo, P. & Rosen, M. R. *Am. J. Physiol.* **242**, H677–H682 (1982).
31. Meyer, K. & Linde, H. in *Venomous Animals and their Venoms* Vol. 2 (eds Buchert, W. & Buckley, E.) 521–552 (Academic, London, 1971).

β -Adrenergic modulation of calcium channels in frog ventricular heart cells

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Adrenergic modulation of calcium channels profoundly influences cardiac function^{1,2}, and has served as a prime example of neurohormonal regulation of voltage-gated ion channels^{1–7}. Channel modulation and increased Ca influx^{2,8,9} are mediated by elevation of intracellular cyclic AMP^{10–17} and protein phosphorylation^{18,19}. The molecular mechanism of the augmented membrane Ca conductance has attracted considerable interest. An increase in the density of functional channels has often been proposed^{20–22}, but there has previously been no direct evidence. Single-channel recordings show that isoprenaline or 8-bromo-cyclic AMP increase the proportion of time individual channels spend open by prolonging openings and shortening the closed periods between openings^{2,23–25}. To look for an additional contribution of changes in the number of functional channels, we applied ensemble fluctuation analysis²⁶ to whole-cell recordings^{27,28} of cardiac Ca channel activity. Here we present evidence that in frog ventricular heart cells β -adrenergic stimulation increases N_F , the average number of functional Ca channels per cell. We also find that isoprenaline slows the time course of both activation and inactivation, and that the enhancement of peak current decreases gradually with greater membrane depolarization.

Frog ventricular cells were chosen because they have a robust β -adrenergic response^{8,22}. We used barium as the current carrier instead of Ca to avoid possible contamination from Ca-activated potassium channels, to block currents through background K channels²⁹ and to improve resolution of the fluctuation analysis by increasing the single-channel current. Series-resistance compensation was used (Fig. 1 legend) and linear leak and capacity

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currents were subtracted to give the traces shown. All experiments were carried out at room temperature (22 °C).

The β -adrenergic agonist isoprenaline strongly enhanced Ca channel current carried by Ba ions (Fig. 1). Figure 1a shows whole-cell currents recorded during depolarizing voltage-clamp pulses from -60 mV to +10 mV. Ca channel current increased from about 0.7 nA in the absence of drug, to about 3.5 nA in the presence of isoprenaline; the effect was fully reversed following washout of the drug. Figure 1b illustrates the time course of the response and the stability of the current amplitude. The increase of Ca channel current was fairly typical; in 25 cells exposed to 0.5 μ M isoprenaline, the whole-cell current increased by a factor of 6.2 ± 0.6 (mean $\pm$ s.e.m.), with individual increases as large as 15-fold.

The current carried by Ca channels (I) depends on three factors: the number of functional channels (N_F), the probability that an individual channel is open (p), and the current carried by an open channel (i). Which of these factors changes with β -adrenergic modulation? To answer this question, we applied ensemble fluctuation analysis as developed by Sigworth²⁶. Ensembles of successively evoked current transients were collected, aligned in time and analysed for the time course of the mean current and the time course of the variance associated with differences in channel activity from one record to the next. Mean and variance records from control and isoprenaline runs are illustrated in Fig. 2a,b. Both the mean current and the variance rose to maximum levels at the end of the depolarizing pulse; like the mean current, the variance increased about fivefold after exposure to isoprenaline. To estimate N_F , p and i for each run, plots of the variance versus mean current (Fig. 2b,c) were fitted by parabolic curves given by $\text{var} = iI - I^2/N_F$. This is the relationship expected for N_F identical, independent, functional channels having one non-zero current level i (ref. 25). The values for N_F and i were obtained by a least-squares curve-fitting procedure. The maximal probability p_{max} was then calculated as $p_{\text{max}} = I_{\text{max}}/(N_F i)$.

In the experiment shown in Fig. 2, N_F increased from ~11,000 to ~37,000 with isoprenaline. The maximal probability increased from 0.40 to 0.58 while the unitary current i remained unchanged at about 0.15 pA. These findings were representative of collected results from a total of eight frog ventricular cells (Table 1). The average value of N_F increased about threefold, while p_{max} increased by about 50%. After recovery from the isoprenaline effect, fluctuation analysis was more difficult because of Ca channel rundown and sweep-to-sweep shifts in baseline. Nevertheless, results from three cells suggested that N_F returned to its pre-isoprenaline level.

One of the most important assumptions of the fluctuation analysis is that the functional channels behave as a single, homogeneous population. Although this is untested, all of our observations seem consistent with this view. When frog ventricular cells are studied with long depolarizing pulses, the Ba current decays as a single exponential over a broad range of potentials. When fluctuation analysis is done on the falling phase of current in these ionic conditions, variance-mean plots are well fitted by a single parabola, with p_{max} near 0.4, just as in analysis of the rising phase in Fig. 2b.

This is the first evidence that the number of functional calcium channels is enhanced by β -adrenergic stimulation, as proposed by Reuter and Scholz²¹ and by others^{20,22}. At the membrane potential level studied here (+10 mV), the overall enhancement of Ca channel current is dominated by the increase in N_F . At other potentials other factors may have a more important role, as discussed below.

Is the Ca channel current simply scaled by β -adrenergic stimulation, or are there changes in time or voltage dependence? Whole-cell recordings allow the kinetics to be studied with good time resolution over a broad range of potentials. Figure 3a shows that the enhancement of inward Ba current in frog ventricular cells is accompanied by a clear slowing in the time course of activation (see also Figs 2a, 4a). The slowing is obvious when the control record is scaled up to match the isoprenaline-

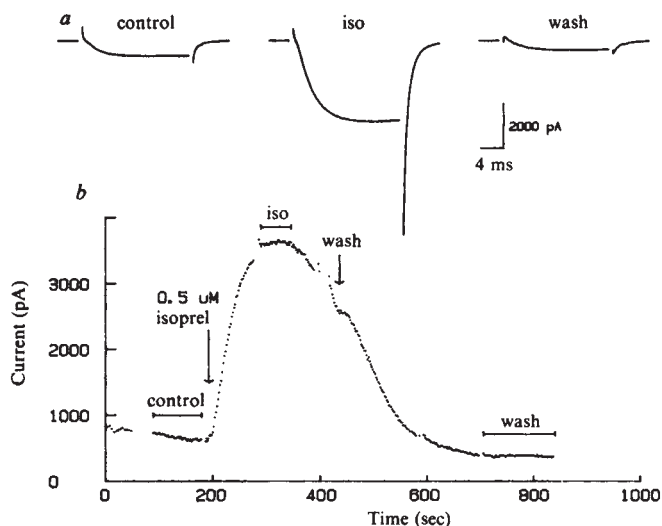


Fig. 1 Increase of Ba currents by isoprenaline. *a*, Currents elicited by a voltage step to +10 mV from a holding potential of -60 mV. Tail currents evoked by a repolarization to -40 mV. Leak and capacity currents (obtained from mirror-image hyperpolarizing pulses, 10 times smaller) have been subtracted, and a 750- μ s period is blanked due to amplifier saturation during capacity transients. Each current shown is the mean current of the series of pulses indicated in *b*. *b*, Time course of isoprenaline response. Current measured at the end of the 20-ms test pulse. 200 μ l of isoprenaline stock solution was squirted into the 1.5-ml chamber to give a final concentration of 0.5 μ M. During the wash, the chamber volume was replaced 20 times with agonist-free solution within the first minute. Note that the current amplitude began to fall before the drug was washed out; in other experiments, current fell back to a stable value near its original level in 5–10 min even though the drug was not washed out, suggesting desensitization or depletion of some intracellular constituent. Cell F04B. Total capacity 75 pF. Series-resistance compensation for 2.2 M Ω out of a measured series resistance of 3.5–3.8 M Ω .

Methods: Single ventricular cells were isolated from bullfrog (*Rana catesbeiana*) hearts following the procedure described by Hume and Giles³⁴ for atrial cells (see also ref. 35). Our procedure included some modifications. After 2–3 h in the final 0.05% collagenase solution, the pieces of ventricle were triturated using a Pasteur pipette. After another 2–3 h of digestion, the cell-containing collagenase solution was diluted 1:4 with (in mM) 1 CaCl₂, 160 NaCl, 5 KCl, 1 MgCl₂, 10 glucose, 5 HEPES pH 7.6. Cells were used 2–24 h after being isolated. For recording whole-cell voltage-clamp currents^{7,28}, cells were placed in solution containing (in mM) 10 CaCl₂, 130 NaCl, 5 KCl, 1 MgCl₂, 10 glucose, 5 HEPES pH 7.6. Electrodes (1–3 M Ω) contained (in mM) 120 CsCl, 10 Cs-EGTA, 5 MgCl₂, 10 HEPES pH 7.5. After formation of a gigaseal and rupture of the cell membrane, the external solution was changed to (in mM) 10 BaCl₂, 135 TEA-Cl, 1 MgCl₂, 10 glucose, 10 HEPES pH 7.65. Pipette series-resistance (measured as the time constant of the uncompensated capacity transient divided by the cell capacity) was 2–8 M Ω , of which 60–80% was compensated.

enhanced trace (lower part of Fig. 3a). The isoprenaline-induced slowing seemed little changed when $[Ba]_o$ was varied between 1 and 30 mM or when membrane potential was varied between -35 and +25 mV. Most of the analysis was done at +10 mV in 10 mM $[Ba]_o$. When the onset of inward current was fitted by a single exponential with a delay (a good approximation for all but the first millisecond after the step), the exponential time constant increased with isoprenaline in 13 out of 13 frog ventricular cells. The time constant was 1.58 ± 0.08 ms (mean $\pm$ s.e.m.) in control preparations and 2.47 ± 0.08 ms in isoprenaline-exposed preparations. Neonatal rat ventricular cells show a similar slowing during exposure to isoprenaline (Fig. 4b), although the enhancement of peak inward current was at most twofold, and typically much less.

Figure 4a compares the peak current-voltage relationship before and after β -adrenergic stimulation, and Fig. 4b plots the

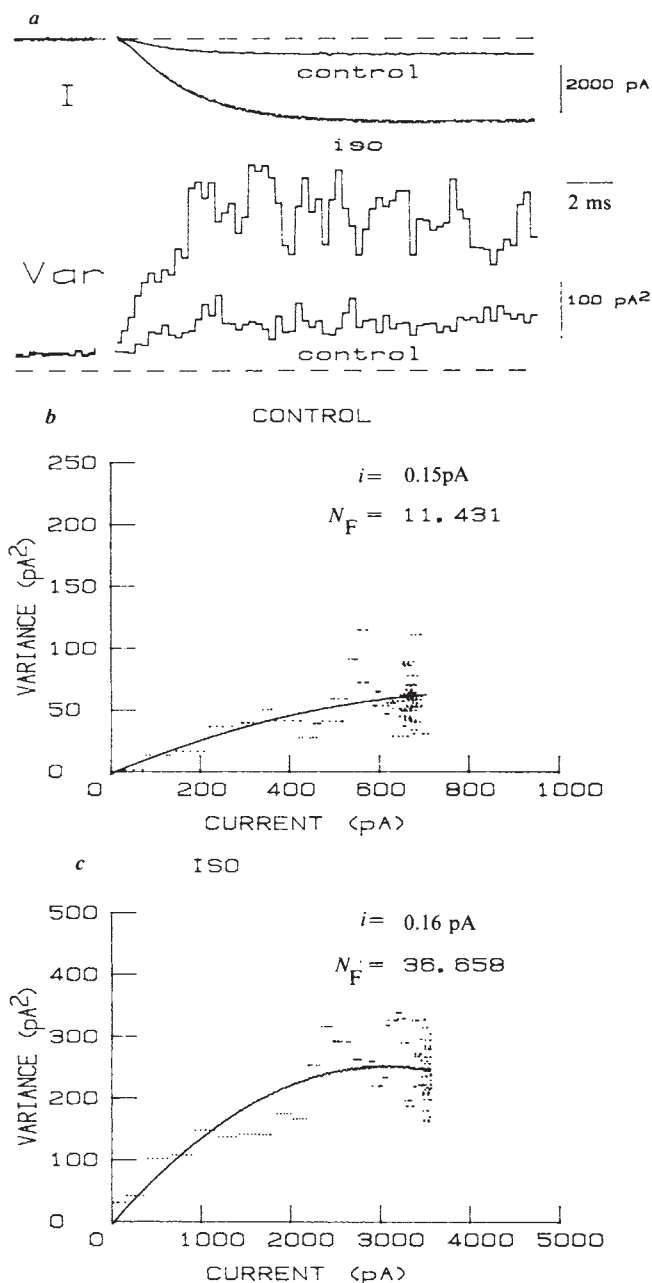


Fig. 2 Fluctuation analysis of Ca channel currents. Same experiment as Fig. 1. Ensembles of currents were elicited by a series of identical pulses to +10 mV (46 in the control and 31 with isoprenaline). Currents were sampled at 30- μ s intervals after being filtered at 3 kHz (4-pole Bessel). Mean current and variance were computed for each time point. To minimize errors from slow drifts in current size, variance was calculated from successive pairs of records and averaged over the pairs; also, the first record of each pair was first scaled by a constant factor to allow for very slow rundown. The first millisecond of the test pulse was omitted from the analysis because the current showed an outward bump during this time; the variance was approximately at its background level. *a*, Mean and variance as a function of time. Mean currents corrected for capacity and leak current. The variance was averaged over 10 successive time points. Dotted lines indicate zero levels. *b*, *c*, Variance plotted against mean current. The background variance (computed from the current at the holding potential) was first subtracted from the variance during the test current.

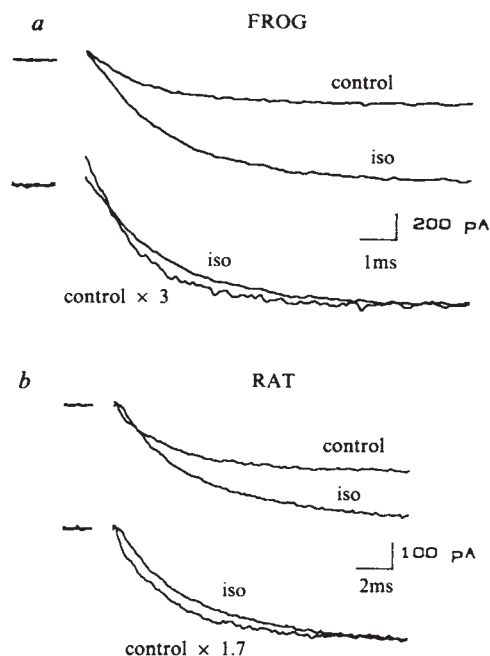


Fig. 3 Effect of isoprenaline on the time course of Ca channel activation. Recordings are shown at original amplification (upper traces in each panel), and with control record scaled up to the same magnitude as the isoprenaline trace (lower traces in each panel). *a*, Frog ventricular cell, 10 mM Ba external solution. Currents evoked by a step depolarization from -60 to +10 mV. Cell F061. *b*, Cultured rat ventricular cell, 10 mM Ba external solution as in *a*. Currents elicited by a depolarizing step from -60 to +30 mV. Cells were cultured from 1-3-day-old rat hearts³⁶. The recordings shown here were made on day 3 after culture. Cell M801.

ratio $I(\text{iso})/I(\text{control})$ as a function of potential. The enhancement of Ca channel current falls from about 11-fold at -40 mV to slightly more than 4-fold at +10 mV (close to the 6-fold average value mentioned earlier). The ratio continues to decrease as the test potential approaches and exceeds the reversal potential for Ca channel current (near +50 mV in the control run). At strongly positive potentials, isoprenaline increases the outward current very little.

The voltage dependence cannot be accounted for by errors due to the series resistance of the pipette or the longitudinal resistance of the cell (see Fig. 4 legend). Interference from other ionic channels would also appear unlikely, in view of the ionic conditions (external Ba and tetraethylammonium (TEA), internal Cs and EGTA), and experiments using the Ca channel blocking drug verapamil (Fig. 4c-e). Verapamil totally abolishes the isoprenaline-enhanced current, whether it is inward (0 mV, Fig. 4c) or outward (+120 mV, Fig. 4e). At the reversal potential in the control run (+50 mV, Fig. 4d), the net driving force for Ca channel current is zero and as expected, neither isoprenaline²¹ nor verapamil³⁰ has any effect. This experiment leaves little doubt that the outward current reflects Ca channel activity alone. Thus, it is notable that β -stimulation hardly increases the peak outward current at all (Fig. 4a,e).

The main effect of isoprenaline in Fig. 4e is a marked slowing of the decay of the outward current. Slowing of inactivation is also manifested by the decay of inward currents accompanying less positive test depolarizations (data not shown), and by the behaviour of inward tail currents evoked by sudden repolarization after test pulses of varying duration, studied in another cell (Fig. 4f,g). In a control run (Fig. 4f), the amplitude of tail currents decreases gradually along with outward current inactivation; after exposure to isoprenaline (Fig. 4g), the amplitude of tails is maintained, like the outward current.

Outward current and tail current appear not to vary concomitantly in their response to isoprenaline: inward tail currents seem to be strongly enhanced at a time (vertical arrow) when

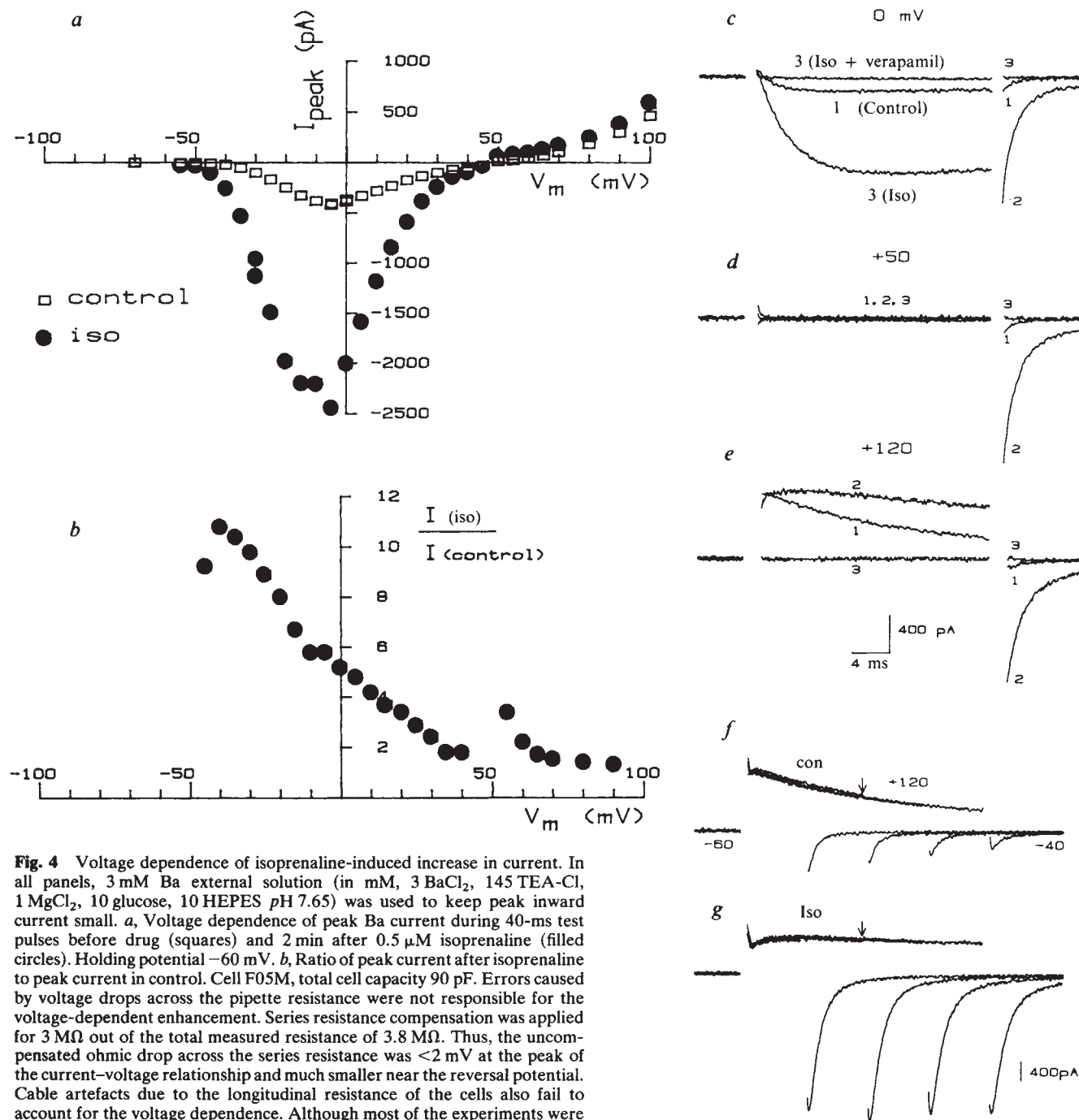


Fig. 4 Voltage dependence of isoprenaline-induced increase in current. In all panels, 3 mM Ba external solution (in mM, 3 BaCl₂, 145 TEA-Cl, 1 MgCl₂, 10 glucose, 10 HEPES pH 7.65) was used to keep peak inward current small. *a*, Voltage dependence of peak Ba current during 40-ms test pulses before drug (squares) and 2 min after 0.5 μM isoprenaline (filled circles). Holding potential -60 mV. *b*, Ratio of peak current after isoprenaline to peak current in control. Cell F05M, total cell capacity 90 pF. Errors caused by voltage drops across the pipette resistance were not responsible for the voltage-dependent enhancement. Series resistance compensation was applied for 3 M Ω out of the total measured resistance of 3.8 M Ω . Thus, the uncompensated ohmic drop across the series resistance was <2 mV at the peak of the current-voltage relationship and much smaller near the reversal potential. Cable artefacts due to the longitudinal resistance of the cells also fail to account for the voltage dependence. Although most of the experiments were done in relaxed cells with cylindrical geometry, we found similar voltage dependence in cells that were deliberately allowed to round up in Ba-TEA solution, even when large, low-resistance pipettes were used and series-resistance voltage drops were <1 mV. *c-e*, Verapamil block of isoprenaline-enhanced currents. Consecutive runs in the absence of drug (trace 1), after exposure to 0.5 μM isoprenaline (trace 2), and after exposure to 50 μM verapamil in the continued presence of isoprenaline (trace 3). Step depolarizations from a holding potential of -60 mV to the test potentials indicated, followed by step repolarization to -40 mV. The capacity transients are blanked for 1.2 ms (*a*), 1.4 ms (*b*) and 2.0 ms (*c*). Noise on records is due to digitization of current traces used for subtraction of linear leak current. Cell F09E. *f, g*, Comparison between behaviour of outward currents during depolarization to +120 mV and inward tail currents following repolarization to -40 mV, before (*f*) and after 0.5 μM isoprenaline (*g*). Inward current evoked by depolarization to 0 mV was increased sevenfold (record not shown). Arrow marks time when outward currents in control and isoprenaline runs are approximately equal. Records are blanked for 720 μs following the onset of the repolarizing step to allow for complete settling of capacity transient. Cell F10G.

the outward current remains essentially unchanged. One possibility is that at strongly positive potentials some or all of the channels show a lower conductance after isoprenaline. We cannot rule out an alternative possibility, however, that the amplitude of the tail is severely underestimated in control because it decays within the settling time of the capacity transient.

The present study shows that β -adrenergic stimulation increases the number of functional channels seen with ensemble fluctuation analysis. Isoprenaline slows the settling time con-

stants for activation and inactivation. The enhancement of Ca channel current is largest at weak depolarizations and smallest at strongly positive potentials. These findings with whole-cell recordings may be compared with studies using cell-attached patch recordings.

The large increase in N_F in frog myocytes seems at variance with unitary recordings in mammalian cells, where changes in the apparent number of channels are seen only rarely^{24,25} or not at all. It is important to realize, however, that fluctuation

Table 1 Fluctuation analysis of the effect of isoprenaline on the factors that determine Ca channel current

Cell	$I_{\max}$ (pA)			i (pA)			$p_{\max}$			N_F		
	Con	Iso	Wash	Con	Iso	Wash	Con	Iso	Wash	Con	Iso	Wash
1P	313	1,508	249	0.09	0.12	0.05	0.41	0.66	0.21	8,664	18,295	22,975
1Q	650	2,672	—	0.12	0.18	—	0.48	0.59	—	11,316	24,794	—
1W	538	4,034	253	0.11	0.14	0.14	0.01	0.58	0.20	—	47,650	8,574
1Z	502	3,325	348	0.09	0.18	0.15	0.10	0.40	0.06	—	45,918	—
3J	496	4,771	—	0.25	0.14	—	0.50	0.10	—	3,961	—	—
4B	708	3,648	419	0.15	0.16	0.08	0.40	0.58	0.11	11,431	36,658	—
4C	335	1,614	207	0.16	0.14	0.05	0.52	0.55	0.07	4,000	20,974	—
5F	792	4,376	220	0.12	0.14	0.15	0.26	0.19	0.43	23,652	—	4,791
6H	683	3,993	—	0.10	0.20	—	0.41	0.72	—	16,791	27,261	—
Mean	557	3,327	299	0.13	0.15	0.10	0.34	0.49	0.18	11,402	31,650	12,113
s.e.m.	±52	±367	±29	0.017	0.008	0.020	0.06	0.07	0.05	2,658	4,480	4,523

Experimental conditions as in Figs 1, 2. N_F was considered indeterminate if the apparent value of $p_{\max}$ fell below 0.20. Average cell capacitance was 57 ± 3.5 pF (mean $\pm$ s.e.m.). Con, control; Iso, isoprenaline.

analysis and unitary current analysis can show different biases in the way they assign changes to N and p . This distinction depends on convention and on the time resolution of the measurements^{1,7,31}. In our fluctuation analysis, N_F refers to the average number of functional channels readily available to open in response to the test depolarization. Since the analysis is based on differences between adjacent records, a channel is not counted toward N_F during any silent periods much longer than the inter-pulse interval. This definition is consistent with the idea that Ca channels continually undergo transitions between functional (phosphorylated) and nonfunctional (dephosphorylated) states¹⁸, and that the number of channels increases during the β -adrenergic response^{2,16,20-22}. N_F is to be distinguished from N_T , the total number of channels in an area of membrane. In a membrane patch, N_T can in principle be determined by counting the maximal number of simultaneous openings during a very long period of recording. We have used N_F to express the idea that channels undergo occasional transitions between functional and nonfunctional conditions, distinct from rapid (millisecond) opening reactions. In single channel recordings, such transitions would presumably give rise to groups of sweeps with no openings.

The slowing of the activation time course was seen in both frog and rat ventricular cells. This might appear surprising, as single-channel analysis in cultured rat ventricular cells has provided convincing evidence that isoprenaline or 8-bromo-cyclic AMP increase the rate constants leading to Ca channel opening^{2,23-25}. In fact, the data of Cachelin *et al.*²⁴ also show a decrease in backward rate constants leading away from the open state. According to our calculations, the net effect predicted from their data is a slowing of activation time course, very much like that seen experimentally (Fig. 3). The agreement leaves open the possibility that after isoprenaline, all channels share the same kinetic properties. This is one of the more critical assumptions of the fluctuation analysis.

The pronounced voltage dependence of the increase in Ca channel current was unexpected because previous studies in multicellular preparations have usually shown equal increases at all potentials (for example, refs 21, 32, 33, but see ref. 8). Differences in the composition of internal or external media are possible factors that cannot be ruled out. The voltage dependence of the β -adrenergic response at relatively weak depolarizations in our experiments seems similar to that seen in single channel recordings (see ref. 2, Fig. 2 and ref. 25, Fig. 5).

The almost complete lack of effect of isoprenaline at strong depolarizations was perhaps our most surprising result. We have

considered two alternative explanations. (1) The lack of increase in outward current could be attributed, as already mentioned, to properties of open Ca channels: for example, isoprenaline might recruit channels which show little or no conductance at strongly positive potentials. (2) The large number of channels that are not readily available for activation at +10 mV might become activated at strongly positive potentials even in the absence of β -stimulation. Distinguishing between these hypotheses would be possible if the approach illustrated in Fig. 4f,g could be extended to the point of resolving the earliest phase of the tail current.

We thank P. Hess, A. P. Fox and J. B. Lansman for helpful discussion and the USPHS, the American Heart Association and the Miles Institute for support.

Received 1 July; accepted 3 November 1983.

1. Tsien, R. W. & Siegelbaum, S. A. in *Physiology of Membrane Disorders* (eds Andreoli, T. E., Hoffman, J. F. & Fanestil, D. D.) 517-538 (Plenum, New York, 1978).
2. Reuter, H. A. *Rev. Physiol.* **41**, 413-424 (1979).
3. Kehoe, J. & Marty, A. A. *Rev. Biophys. Bioengng* **9**, 437-465 (1980).
4. Nicoll, R. A. *Trends Neurosci.* **5**, 369-374 (1983).
5. Reuter, H. *Nature* **301**, 569-574 (1983).
6. Tsien, R. W. A. *Rev. Physiol.* **45**, 341-358 (1983).
7. Siegelbaum, S. A. & Tsien, R. W. *Trends Neurosci.* **6**, 307-313 (1983).
8. Vassort, G. *et al.* *Pflügers Arch. ges. Physiol.* **309**, 70-81 (1969).
9. Brown, H. F., McNaughton, P. A., Noble, D. & Noble, S. J. *Phil. Trans. R. Soc. B270*, 527-537 (1975).
10. Tsien, R. W., Giles, W. & Greengard, P. *Nature new Biol.* **240**, 181-183 (1972).
11. Meinert, T., Nawrath, H. & Scholz, H. *Naunyn-Schmiedeberg's Archs Pharmacol.* **279**, 327-338 (1973).
12. Watanabe, A. M. & Besch, H. R. *Circulation Res.* **35**, 316-324 (1974).
13. Reuter, H. *J. Physiol., Lond.* **242**, 429-451 (1974).
14. Vogel, S. & Sperelakis, N. *J. molec. cell. Cardiol.* **13**, 51-64 (1981).
15. Trautwein, W., Taniguchi, J. & Noma, A. *Pflügers Arch. ges. Physiol.* **392**, 307-314 (1982).
16. Nargeot, J., Nerbonne, J. M., Engels, J. & Lester, H. A. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
17. Tsien, R. W. *Adv. Cyclic Nucleotide Res.* **8**, 363-420 (1977).
18. Osterrieder, W. *et al.* *Nature* **298**, 576-578 (1977).
19. Rinaldi, M. L., Capony, J.-P. & Demaille, J. G. *J. molec. cell. Cardiol.* **14**, 279-289 (1982).
20. Sperelakis, N. & Schneider, J. *Am. J. Cardiol.* **37**, 1079-1085 (1976).
21. Reuter, H. & Scholz, H. *J. Physiol., Lond.* **264**, 49-62 (1977).
22. Niedergerke, R. & Page, S. *Proc. R. Soc. B197*, 333-367 (1977).
23. Reuter, H., Stevens, C. F., Tsien, R. W. & Yellen, G. *Nature* **297**, 501-504 (1982).
24. Cachelin, A. B., de Peyer, J. E., Kokubun, S. & Reuter, H. *Nature* **304**, 462-464 (1983).
25. Reuter, H., Cachelin, A. B., de Peyer, J. E. & Kokubun, S. *Cold Spring Harb. Symp. quant. Biol.* **48** (in the press).
26. Sigworth, F. J. *J. Physiol., Lond.* **307**, 97-129 (1980).
27. Hamill, O., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch. ges. Physiol.* **391**, 85-100 (1981).
28. Fenwick, E. M., Marty, A. & Neher, E. *J. Physiol., Lond.* **331**, 599-635 (1982).
29. Cranefield, P. F. & Gadsby, D. C. *J. Physiol., Lond.* **318**, 34-35P (1981).
30. Lee, K. S. & Tsien, R. W. *Nature* **302**, 790-794 (1983).
31. Sigworth, F. J. *J. Physiol., Lond.* **307**, 131-142 (1980).
32. Noma, A., Kotake, H. & Irisawa, H. *Pflügers Arch. ges. Physiol.* **388**, 1-9 (1980).
33. Kass, R. A., Wiegand, S. E. *J. Physiol., Lond.* **322**, 541-558 (1982).
34. Hume, J. R. & Giles, W. *J. gen. Physiol.* **78**, 19-42 (1981).
35. Tarr, M., Trank, J. W. *Experientia* **32**, 338-339 (1976).
36. Hargy, I., Hoover, F. & Farley, B. *Meth. Enzym.* **32**, 740-745 (1974).

Reversible shape change of Triton-treated erythrocyte ghosts induced by Ca^{2+} and Mg-ATP

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The shape of the human erythrocyte depends on intracellular ATP content^{1,2} and echinocytic erythrocyte ghosts obtained in the presence of Mg-ATP acquire a diskocytic shape³⁻⁵ after incubation at 37 °C; however, agreement is lacking about the molecular basis of the shape changes. The suggestion that phosphorylation of cytoskeletal structures underlying the membrane is involved^{5,6} has been disputed and alternative explanations based on lipid bilayer theory^{7,8} and metabolism of phospholipid^{9,10} have been proposed. Recently, we re-examined the effect of ATP on the shape of ghosts and found that it consists of two distinct steps¹¹. We have, therefore, now examined the effect of physiological concentrations of Ca^{2+} and ATP on the cytoskeleton of the erythrocyte membrane directly using Triton-treated ghosts which had lost this permeability barrier. Our findings suggest that a noncovalent effect of ATP on the cytoskeleton is a prerequisite for shape change.

Untreated ghosts were spherical in 10-mM Tris-Cl buffer. On addition of 10^{-7} – 10^{-6} M Ca^{2+} buffer pH 7.4, they crenated immediately, but rapidly changed into diskocytes on further addition of 1 mM Mg-ATP (ref. 11). A similar shape change was also observed when Triton-treated ghosts were used. It was clear that these Triton-treated ghosts were not intact for the following reasons: first, the membrane preparation showed decreased amounts of band 3 and 4.2 in SDS-polyacrylamide gel electrophoresis (PAGE) patterns¹² and these bands were recovered in the supernatant instead of the pellet (Fig. 1). Second, Triton-treated ghosts did not shrink in hypertonic sucrose solutions (0.6 M, 0.8 M), demonstrating the absence of an osmotic barrier. Third, in dark-field microscopy, the surface of untreated ghost membranes was smooth, while that of Triton-treated ghosts membranes was rough (Fig. 2); the rough surface seemed to consist of a large number of small particles, as opposed to spicules or small projections. Electron microscopy after negative staining showed a number of small vesicles (diameter

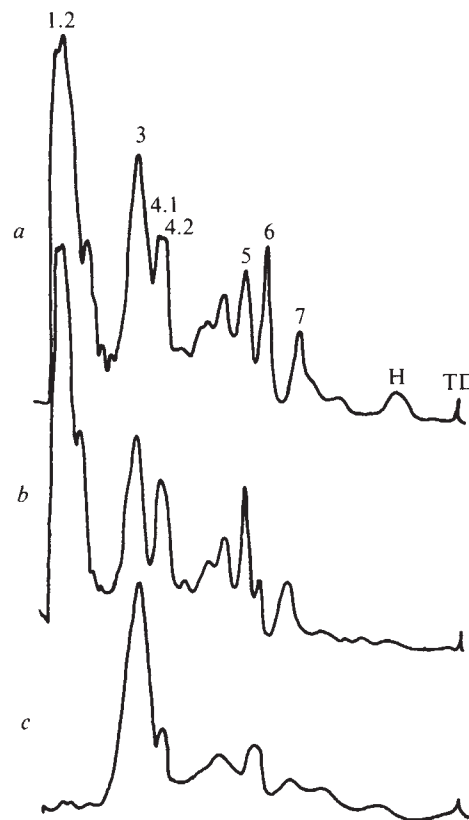


Fig. 1 Densitometric scan of Coomassie blue-stained SDS polyacrylamide gels (5.6%). 10 mM Tris ghosts (a), pellet (b), supernatant fractions (c) derived from 10 mM Tris ghosts after 0.5% Triton treatment. Gels were run and bands numbered accordingly to Fairbanks *et al.*¹³. In each case, 40 µg was applied.

Methods: Human erythrocytes obtained from ACD blood (fresh or preserved for about 4–10 weeks) were washed with physiological saline and haemolysed by 1:50 dilution with 10-mM Tris-HCl buffer, pH 7.4, or with 20-mM Tris-maleate buffer containing 1×10^{-3} M CaCl_2 , and 1×10^{-3} M EGTA, pH 7.4 (10^{-6} M Ca^{2+} buffer), at 0 °C, and centrifuged at 18,000 r.p.m. for 20 min at 4 °C. When long-preserved blood was used, the membranes were preincubated for 20 min at 37 °C in 2-mM Mg-ATP solution (pH 7.4) (or with 2-mM Mg-ATP in 10^{-6} M Ca^{2+} -buffer). Membranes (converted to diskocytic form by preincubation) were washed with 1 mM MgCl_2 at 4 °C (18,000 r.p.m., 20 min). This transformed them to spheres as previously described¹¹. Ghosts from fresh cells or pretreated ghosts from preserved blood were mixed with 10 volumes of 1-mM MgCl_2 (pH 7.4) containing 0.5% Triton X-100. After 5 min at 0 °C, the suspensions were centrifuged at 6,000 r.p.m. for 20 min.

0.08–0.22 µm) and a filamentous network (Tsukita *et al.*, unpublished observation). Fourth, 1% Triton could be used instead of 0.5% Triton, although 1% Triton-treated ghosts were less readily visible.

Triton-treated ghosts were larger when prepared with 10-mM Tris-Cl buffer instead of 10^{-6} -M Ca^{2+} -buffer, but shrank for a period of several minutes when suspended in Ca^{2+} buffer (10^{-7} – 10^{-6} M) (Figs 2, 3) and became the same size as the Triton-treated ghosts prepared in 10^{-6} -M Ca^{2+} buffer. In fact, shrunken ghosts were spherical and swollen ghosts were diskocytic. These shrunken Triton-treated ghosts swelled on addition of 1 mM Mg-ATP at both 6 °C and room temperature within several minutes (Figs 2, 3) and shrank again after being washed with 1 mM MgCl_2 (pH 7.4) containing 10^{-6} M Ca^{2+} at both 6 °C and room temperature in the pH range, 6.8–7.8. The same shape change was also observed when the incubated Triton-treated ghosts were fixed for 30 min with 1% glutaraldehyde buffered with 10 mM imidazole-HCl (pH 7.4) at 0 °C or at room tem-

Table 1 Effect of nucleotides and EGTA on the diameters of Triton-treated erythrocyte ghosts shrunk by 10^{-6} M Ca^{2+}

Addition	Diameter µm, n = 30
None	4.29 ± 0.47
1 mM MgCl_2 + 1 mM EGTA	7.53 ± 0.59
1 mM Mg-ATP	6.37 ± 0.63
1 mM Mg-ADP	4.52 ± 0.60
1 mM Mg-GTP	4.51 ± 0.72
1 mM Mg-UTP	4.85 ± 0.55
1 mM Mg-ITP	4.50 ± 0.34

The diameters of Triton-treated ghosts were measured in dark-field light micrographs. Average diameter of ghosts was determined in randomly selected fields and 30 Triton-treated ghosts were measured for each suspension. The preparation of 10^{-6} M Ca^{2+} buffer is described in Fig. 3 legend. Triton-treated ghosts in 10^{-6} M Ca^{2+} buffer were mixed with the indicated solutions at pH 7.4, 0 °C and photographs were taken after 10 min at room temperature. When these Triton-treated ghosts were fixed with 1% glutaraldehyde and then 2% osmium tetroxide the results obtained were essentially the same. Both fixatives were dissolved in 10 mM imidazole-HCl and Ca^{2+} EGTA buffer instead of 20 mM Tris-maleate and Ca^{2+} EGTA buffer.

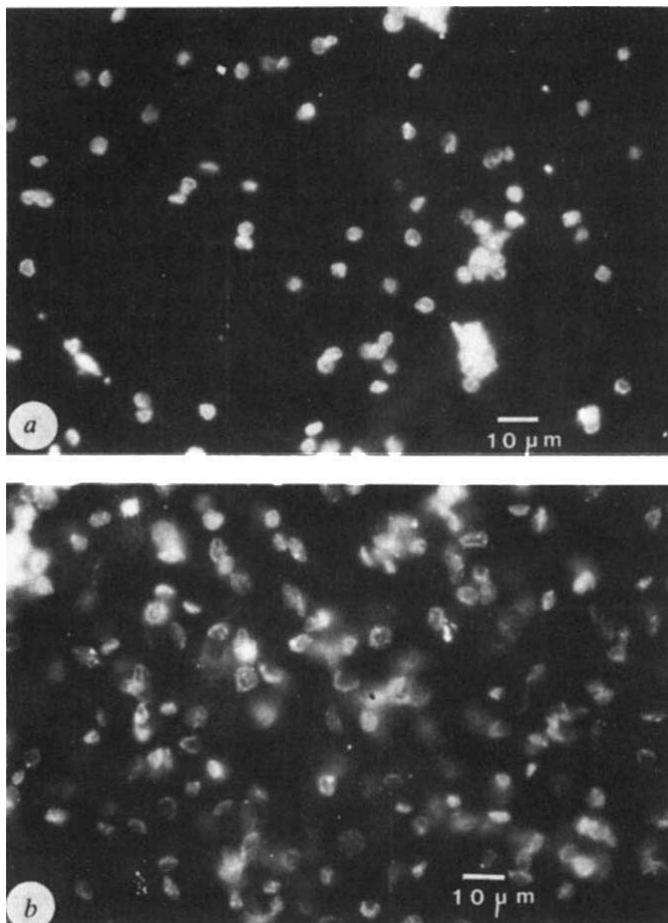


Fig. 2 Dark-field light micrographs of Triton-treated ghosts. The ghosts were prepared with 10 mM Tris-Cl buffer (pH 7.4), incubated in 2 mM Mg-ATP (pH 7.4) at 37°C for 30 min, washed with 1 mM MgCl₂ (pH 7.4) and treated with 0.5% Triton at 0°C for 5 min. 10 μl of Triton-treated ghosts was dropped into 170 μl of 10⁻⁶ M Ca²⁺-buffer at 0°C (a) and 20 μl 10-mM Mg-ATP in 20 mM Tris-maleate buffer (pH 7.4) was added at 0°C (b). The shapes of ghosts and Triton-treated ghosts were observed under a dark-field light microscope (Nikon Model S). In 10⁻⁶ M Ca²⁺-buffer, Triton-treated ghosts appear to be rough-surfaced small spheres, but on addition of Mg-ATP, they appear to be rough-surfaced disks. These rough-surfaced ghosts seem to be quite different from intact ghosts. The Ca²⁺-buffer used is described in Fig. 3.

perature and then post-fixed with 2% osmium tetroxide for 30 min at the same temperature. In these experiments, 10 mM imidazole-HCl, Ca²⁺ EGTA buffer was used instead of 20 mM Tris-maleate buffer¹³. Since fixation by 0.5–2% glutaraldehyde was not completed in 30 min at 0°C, we could not determine the exact time required for the shape change. Mg-pyrophosphate, Mg-UTP, Mg-GTP, Mg-ITP, Mg-adenylyl-imidodiphosphate (AMPPMP) and Mg-adenylyl-(β-γ)methylene diphosphate (AMPPCP) were not effective (Table 1) in reversing Ca²⁺-mediated shrinkage. In the absence of ATP, low concentrations of Ca²⁺ induced shrinkage of the Triton-treated ghosts: the $K_{0.5}$ for Ca²⁺ was about 10⁻⁷ M, in contrast with the relatively high value of 3×10⁻⁶ M in the presence of ATP (Fig. 3). Triton-treated ghosts, like untreated ghosts, could change shape reversibly depending on Mg-ATP in the presence of 10⁻⁶ M Ca²⁺.

The results described above showed that crenation or disk formation of ghosts occurred in hypotonic solutions with low concentrations of Ca²⁺ or Mg-ATP, respectively, and the shrinking or swelling, respectively, of Triton-treated ghosts occurred in the same conditions. Therefore, crenation of ghosts corresponds to the shrinkage of Triton-treated ghosts, and disk formation of ghosts, to the swelling of Triton-treated ghosts. When isotonicity was restored by adding KCl or NaCl, Triton-treated ghosts shrank immediately and did not respond to either Ca²⁺

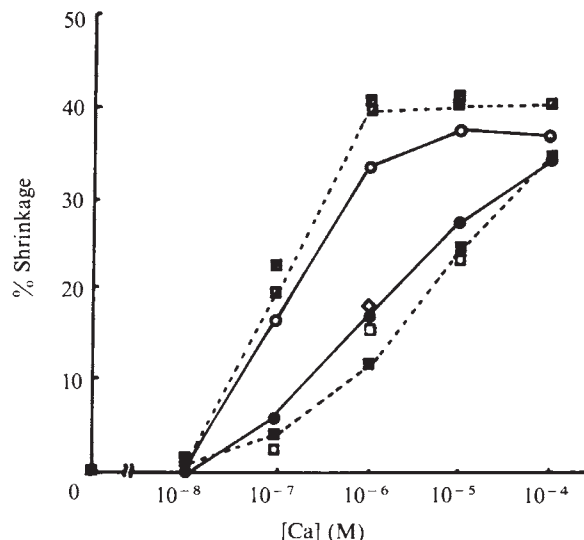


Fig. 3 Effect of Mg-ATP on the shrinkage of Triton-treated erythrocyte ghosts. A 10-μl aliquot of Triton-treated ghosts prepared with 10-mM Tris-Cl buffer and 1-mM MgCl₂ was mixed with 170-μl Ca²⁺ buffer containing the indicated concentration of Ca²⁺. ○, Triton-treated ghosts from fresh cells; ●, Triton-treated ghosts from fresh cells on addition of 20 μl 10-mM Mg-ATP; □, Triton-treated ghosts from preserved cells (10 weeks); ■, Triton-treated ghosts from preserved cells preincubated with adenine and inosine⁵; ▤, Triton-treated ghosts from preserved cells preincubated with 2-mM Mg-ATP after haemolysing and washing, Triton-treated ghosts from preserved cells preincubated with 2 mM Mg-ATP in the presence of 1 mM Mg-ATP; ■, Triton-treated ghosts from preserved cells in the presence of 1 mM Mg-ATP.

Methods: Thirty Triton-treated ghosts were measured as described in Table 1 legend. Shrinkage (%) = $(c - a/c) \times 100$, where c = diameter of Triton-treated ghosts in 1 mM Mg-EGTA solution (pH 7.4) a = diameter of Triton-treated ghosts in the indicated Ca²⁺ buffer. Ca²⁺ EGTA buffer was prepared with 20 mM Tris-maleate buffer (pH 7.4) containing 0.3 ml 1 mM CaCl₂ and various amounts of EGTA as follows in a total volume of 3 ml: Ca²⁺ 1×10⁻⁸ M, 10 mM EGTA 0.633 ml; Ca²⁺ 1×10⁻⁷ M, 1 mM EGTA 0.957 ml; Ca²⁺ 1×10⁻⁶ M, 1 mM EGTA 0.414 ml; Ca²⁺ 1×10⁻⁵ M, 1 mM EGTA 0.366 ml; Ca²⁺ 1×10⁻⁴ M, 1 mM EGTA 0.60 ml (ref. 20).

or Mg-ATP, agreeing with results reported by Johnson *et al.*¹⁴. Since shrinking and swelling of Triton-treated ghosts were fast, even at 6°C, and Triton-treated ghosts lost the permeability barrier, Mg-ATP must act on the cytoskeleton directly and the effect is essentially different from phosphorylation of lipid and protein. The effect of Mg-ATP might not be due to removal of Ca²⁺, because the shape change occurred even in the presence of 3×10⁻³ M EGTA + 3×10⁻³ M Ca²⁺, although a nominally equimolar mixture of Ca²⁺ and EGTA will have an indeterminate Ca²⁺ concentration. The sensitivity to Ca²⁺ presumably does not depend on calmodulin because most of calmodulin is lost in the membranes during preparation of ghosts¹⁵ and calmodulin inhibitors¹⁶ (chlorpromazine, trifluoperazine and W-7) did not inhibit this Ca²⁺-induced shrinking (not shown).

Figure 3 shows that phosphorylation of the Triton-treated ghosts seems to be correlated with the Ca²⁺-induced shrinking and ATP-induced fast swelling. Both Triton-treated ghosts from fresh cell ghosts without preincubation and from preserved cell ghosts preincubated with Mg-ATP (Fig. 3) were highly sensitive to Ca²⁺ in the absence of Mg-ATP. On addition of Mg-ATP, both of them became relatively insensitive to Ca²⁺ (Fig. 3). However, Triton-treated ghosts from preserved cells without preincubation were not highly sensitive to Ca²⁺ with or without the addition of Mg-ATP (Fig. 3). After alkaline phosphatase treatment of fresh cell ghosts⁶, their Triton-treated ghosts became less sensitive to Ca²⁺ ions, and then they recovered Ca²⁺ sensitivity by re-incubation with 2-mM Mg-ATP for 20 min at 37°C (Table 2).

Table 2 Ca^{2+} sensitivity of Triton-treated erythrocyte ghosts

Source	Pretreatment of intact ghosts	Addition of Mg-ATP	Shape	Ca^{2+} sensitivity
Fresh	None	+	Swell	Low
		-	Shrink	High
	Phosphatase*, wash	+	Swell	Low
		-	Swell	Low
	Phosphatase + P_i^* , wash	+	Swell	Low
		-	Shrink	High
Preserved	Phosphatase, wash then reincubated with Mg-ATP	+	Swell	Low
		-	Shrink	High
	Heat-denatured phosphatase	+	Swell	Low
		-	Shrink	High
	None	+	Swell	Low
		-	Swell	Low
	Preincubation† with Mg-ATP	+	Swell	Low
		-	Shrink	High

* Ghosts in a haematocrit of 50% in 10-mM Tris-Cl buffer (pH 7.4) incubated with 0.2 mg per ml alkaline phosphatase (*Escherichia coli*, Sigma III R) with and without 10-mM P_i at 4 °C for 20 min as described Birchmeier and Singer⁶.

† Preincubation was carried out with 2 mM Mg-ATP at 37 °C for 20 min.

‡ K_m values for Ca^{2+} were roughly 10^{-7} M (High) and 10^{-6} M (Low).

We expected that preserved cell ghosts were dephosphorylated, but that fresh cell ghosts, as well as old cell ghosts treated with Mg-ATP, could be phosphorylated. Direct measurement supported this. The amounts of phosphate in lipid and protein of Triton-treated ghosts were determined after extracting extensively with chloroform, methanol and 1.2 M HCl (10:10:1). Although the phosphate content of lipid was almost constant (about 17 μg per mg protein), the phosphate content of protein was gradually decreased from 0.63 μg per mg protein to 0.25 μg per mg protein during preservation (1–13 weeks). Dephosphorylation of membrane protein seemed to occur during storage. Phosphorylation of membrane protein with 1 mM Mg-ATP³² was reported by various investigators^{17,18}. In this experiment, preincubation with 1-mM Mg- $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (specific radioactivity 1.3×10^7 c.p.m. per μmol of ATP) at 37 °C for 30 min resulted in labelling of the ghosts with ^{32}P ($\sim 2.6 \times 10^4$ c.p.m.), and about 85% of this ^{32}P (about 2.3×10^4 c.p.m.) was removed by Triton treatment. The remaining 15% of the label was present in the Triton-treated ghosts (122 pmol per mg protein), although 50% of phospholipids was removed after Triton treatment. After extensive extraction with acid/chloroform/methanol the ^{32}P content of the membrane was 36 pmol per mg protein. Since the value, 122 pmol per mg, is so small compared with the total ghost phospholipid, 1×10^6 pmol per mg protein, it is not likely that the phosphorylation of lipid is more important than phosphorylation of protein.

We consider that this shrinking and swelling of Triton-treated ghosts involve effects produced on the cytoskeleton at physiological concentrations of Ca^{2+} and ATP and that phosphorylation of cytoskeleton and a noncovalent effect of ATP on cytoskeleton are the basis of this phenomenon.

Received 21 June; accepted 3 November 1983.

1. Nakao, M., Nakao, T. & Yamazoe, S. *Nature* **187**, 945–946 (1969).
2. Nakao, M., Nakao, T., Yamazoe, S. & Yoshikawa, H. *J. Biochem.* **49**, 487–492 (1961).
3. Nakao, M., Nakao, T., Tatibana, M. & Yoshikawa, H. *J. Biochem.* **47**, 694–695 (1960).
4. Palck, J., Stewart, G. & Lionetti, G. *Blood* **44**, 483–497 (1974).
5. Sheetz, M. P. & Singer, S. J. *Cell Biol.* **73**, 638–646 (1977).
6. Birchmeier, W. & Singer, S. J. *J. Cell Biol.* **73**, 647–659 (1977).
7. Sheetz, M. P. & Singer, S. J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4457–4461 (1974).
8. Sheetz, M. P., Painter, R. G. & Singer, S. J. *J. Cell Biol.* **70**, 193–203 (1976).
9. Quist, E. E. & Reece, K. L. *Biochem. biophys. Res. Commun.* **95**, 1023–1030 (1980).
10. Fairbanks, G., Patel, V. & Dino, J. *Scand. J. clin. lab. Invest.* **41**, 139–144 (1981).

11. Jinbu, Y., Nakao, M., Otsuka, M. & Sato, S. *Biochem. biophys. Res. Commun.* **112**, 384–390 (1983).
12. Yu, J., Fischman, D. A. & Steck, T. L. *J. supramolec. Struct.* **1**, 233–248 (1973).
13. Hellewell, S. B. & Taylor, D. L. *J. Cell Biol.* **83**, 633–648 (1979).
14. Johnson, R. M., Taylor, G. & Meyer, D. M. *J. Cell Biol.* **86**, 371–376 (1980).
15. Jarrett, H. W. & Penniston, J. T. *J. Biol. Chem.* **253**, 4683 (1978).
16. Nelson, G. S., Andrews, M. L. & Karnovsky, M. J. *J. Cell Biol.* **96**, 730–735 (1983).
17. Harris, H. W. Jr & Lux, S. E. *J. Biol. Chem.* **255**, 11512–11520 (1980).
18. Imhof, B. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 3264–3268 (1980).
19. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. *Biochemistry* **10**, 2606–2617 (1971).
20. Ogawa, Y. *J. Biochem.* **64**, 255 (1968).

Viscoelastic properties of erythrocyte membranes in high-frequency electric fields

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The high deformability of erythrocytes which is essential for their transport through the capillaries depends critically on their discoid shape and on the elasticity of the plasma membrane, which may be determined by interactions of the cytoskeleton, the lipid/protein leaflet and the glycocalyx. Although techniques exist for measurement of the static elastic properties of erythrocytes, the cells are continuously deformed *in vivo*, the stress varying within periods of a few seconds. Thus dynamic elastic behaviour is essential for their physiological function. We present here a novel means of measuring the dynamic elastic constants of the red cell based on the transient deformation of individual cells in an inhomogeneous high-frequency (HF) electric field. By microscopy it is possible to record cellular elongations as small as 200 nm occurring within time scales of 1 ms. A main advantage is that the cellular response is linear and thus can be more readily interpreted theoretically. We have observed a creep function consisting of two exponentials with response times of 0.1 s and 1 s, which can be described in terms of a simple viscoelastic model. A remarkable temperature dependence of the membrane elasticity between 25 °C and 15 °C is observed for freshly drawn cells but not for trypsinized ones.

Blood was freshly drawn from the fingertip and diluted in 1 mM phosphate buffer (pH 7.5), containing 300 mM D-mannitol and 5 mM glucose. For the trypsin treatment the cells were incubated at 37 °C for 30 min after addition of 10 p.p.m. trypsin and 4 p.p.m. EDTA to the solution. The cell suspension was placed between two electrodes with sharp edges (Fig. 1a). Due to the different conductivities of the cytoplasm ($\sigma_i = 10^{-1} \text{ m}^{-1}$) and the external medium ($\sigma_e = 10^{-2} \text{ m}^{-1}$) the behaviour of the cells in the inhomogeneous field depends on the frequency, f , in the following way¹. At $f > f_2 (\sim 10^7 \text{ Hz})$ both media behave like insulators and the cells swim freely. At $f_1 (\sim 10^5 \text{ Hz}) < f < f_2 (\sim 10^7 \text{ Hz})$ the conductivity of the cytoplasm can no longer be neglected. This leads to the accumulation of opposite charges at the two ends of the cell opposing the electrodes as indicated in Fig. 1b. Due to this so-called Maxwell-Wagner polarization¹ the cell behaves as an electric dipole and couples to the electric field. At $f < f_1$ the cells start to rotate about an axis parallel to the edges of the electrodes. In contrast to Holzapfel *et al.*² we observe the rotation of individual cells³.

For the experiments the frequency is adjusted to a certain value within the second frequency regime. The cells are attracted to one of the electrodes by a bias field of 10^4 V m^{-1} . For the deformation the amplitude is suddenly increased to 2×10^4 – 10^5 V m^{-1} for 2.5 s and is then reset to the original value. The time-dependent elongation of the cell—the so-called creep function, $C(t)$ —as well as the decay in the original shape, are measured microscopically as shown in Fig. 1.

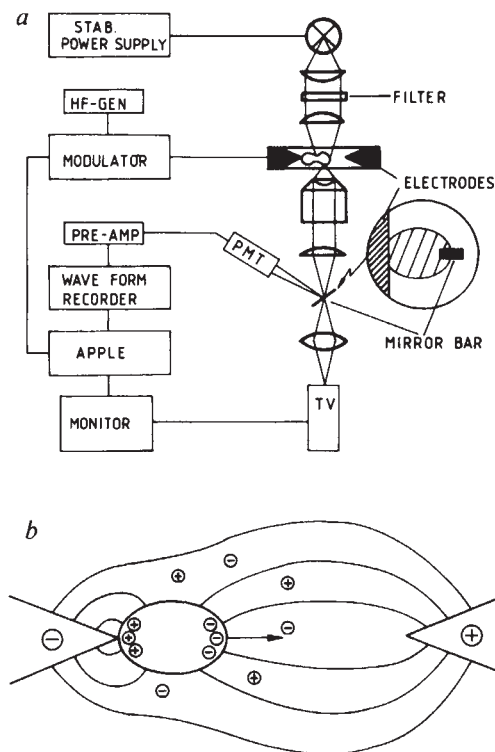


Fig. 1 *a*, Schematic view of an experimental device used to measure the creep ($C(t)$) and decay ($R(t)$) functions after application of a high-frequency field jump. The electrodes are made of commercial razor blades. The curvature of their edges is about $0.5 \mu\text{m}$ and the electrode distance is $50 \mu\text{m}$. *b*, Schematic view of electric field lines and charge accumulation within the cell. This is due to the Maxwell-Wagner polarization which arises as a consequence of the different conductivities of the cytoplasm ($\sigma_i = 10^{-1} \text{m}^{-1}$) and that of the outside medium ($\sigma_o = 10^{-3} \Omega^{-1} \text{m}^{-1}$). **Methods:** Deformation was achieved in the frequency range 5×10^5 – 2×10^6 Hz if the conductivity of the outside medium was adjusted to $\sigma_o = 10^{-2} \Omega^{-1} \text{m}^{-1}$. The cells were fixed to one of the electrodes by a bias voltage of 1 V. For the transient deformation, field jumps of 2–5 V amplitude and ~2.5 s duration were applied. The HF field was generated by a sine generator equipped with a computer-controlled amplitude modulation. A modified Zeiss Axiomat microscope and a Zeiss oil immersion objective (type Planapo 1.3; oil Polox/017) ($\times 100$) were used. Bright-field illumination at 410 nm was supplied by a halogen lamp driven by a stabilized power supply. An image of the object was projected onto a TV camera, which allowed certain cells to be selected and observed during measurement. The deformation was measured with a bar-like mirror, deposited on a glass plate, positioned in the focal plane of the objective. The light reflected by this mirror was focused on a photomultiplier (RCA 931 b). If the cell is elongated, the light intensity at the photomultiplier is decreased, the decrease in intensity being proportional to the elongation of the cell. The photomultiplier output is first amplified and then fed into a waveform recorder (Biomation model 1015). From there, the signal is transferred to an Apple microcomputer for signal averaging up to 256 events.

Figure 2 shows typical response curves for a freshly drawn cell (Fig. 2*a*) and for a cell treated with trypsin. Both the creep ($C(t)$) and the decay ($R(t)$) functions exhibit a fast and a slow component. $C(t)$ can be well represented by

$$C(t) = A_0 - A_1 e^{-t/\tau_1} - (A_0 - A_1) e^{-t/\tau_2} \quad (1)$$

A similar equation holds for $R(t)$. A quantitative evaluation of the experimental curves shows that the time dependencies of $C(t)$ and $R(t)$ are slightly different. This is due to the electric field inhomogeneity through which the force decreases with the degree of elongation (Fig. 3).

As shown in Fig. 2*a*, the maximum amplitude A_0 at 13°C is smaller by a factor of 5 than at 30°C . This effect has been observed for 9 out of 10 cells. It is reversible if the temperature is increased and decreased several times. Obviously, this remarkable temperature dependence is not observed for trypsinized cells (Fig. 2*b*). For a first quantitative evaluation of the viscoelastic response curves, it is helpful to introduce a viscoelastic model. As $C(t)$ and $R(t)$ consist of a fast and a slow process, the model of Fig. 3*b* is used⁴. A different model proposed by Rand⁵ is adapted to the behaviour of cells at large deformations and long time scales. Our model is characterized by four parameters: two spring constants (μ_1, μ_2) and two viscosities (γ_1, γ_2) which can be determined as follows.

The amplitudes and relaxation times of equation (1) are expressed in terms of the four parameters of the model and the force F_0 by solving the differential equations of the model (Fig. 3) using standard procedures. The parameters are then determined by fitting the theoretical functions $C(t)$ (and $R(t)$) to the experimental response curves. This was done using an Apple computer by the Newton procedure. Finally, μ_1, μ_2, γ_1 and γ_2 are determined in units of the force F_0 . Since F_0 cannot be measured yet, it has been estimated in the following way. For $f_1 < f < f_2$ the electric field, E_i , inside the cell is small compared with that at the outside, E_o , since $\sigma_i \gg \sigma_o$. F_0 is obtained by integrating the Maxwell tension over the surface area A of the cell and by time averaging. It is given approximately by: $F_0 \sim 1/4 \epsilon_o \epsilon \times E^2 A$. For $\epsilon = 80$, $A = 10 \mu\text{m}^2$ and $E_a \sim E_o = 10^5 \text{Vm}^{-1}$ one obtains $F_0 \sim 2 \times 10^{-11} \text{N}$. The absolute values of the parameters in Table 1 hold for this value of F_0 . The spring constant μ_1 is directly given by the maximum amplitude (which was adjusted to $\sim 1 \mu\text{m}$) according to

$$\mu_1 = A_0 F_0 = 2 \times 10^{-5} \text{N m}^{-1} \quad (2)$$

μ_1 . A measure of the elastic modulus of the cell, is of the same order of magnitude as the elastic shear modulus ($7 \times 10^{-6} \text{N m}^{-1}$ at 25°C) obtained with the micropipette method^{6,8}.

To account for the two exponentials of $C(t)$, the model must contain two dash pots and two springs. A manifold of other arrangements would account for the response function. The model was chosen for its simplicity. To describe the membrane viscoelasticity in terms of extensive elastic constants it is necessary to determine the change of the cellular shape quantitatively. Following Hochmuth *et al.*⁷ and Waugh⁹, an upper limit on the surface viscosity of the plasma membrane may be estimated according to $\eta = \mu \times \tau$. The value of $\gamma = 3 \times 10^{-6} \text{Ns m}^{-1}$ is again of the same order of magnitude as that obtained with the micropipette technique: $7 \times 10^{-7} \text{Ns m}^{-1}$ (refs 7, 8). It is, however, two orders of magnitude higher than the surface viscosity of the lipid protein bilayer of the red cell as obtained from lateral diffusion studies^{9,10}. This shows that the membrane

Table 1 Amplitudes (A_0, A_1), relaxation times (τ_1, τ_2), spring constants (μ_1, μ_2) and viscosities (γ_1, γ_2) for normal and trypsinized cells.

Type of cell	Temperature ($^\circ\text{C}$)	A_0 (μm)	A_1	τ_1 (s)	τ_2	μ_1 (10^{-5}N m^{-1})	μ_2 (10^{-5}N m^{-1})	γ_1 ($10^{-6} \text{N s m}^{-1}$)	γ_2
Normal	30	1	0.3	0.16	0.9	1.0	0.74	3.7	2.9
	16	0.2	—	—	—	—	—	—	—
Trypsinized	30	1	0.64	0.1	0.9	1.0	0.48	1.7	2.4
	14	1.1	0.45	0.1	0.9	1.1	1.25	2.6	3.8

The absolute values hold for an external force of $F_0 = 2 \times 10^{-11} \text{N}$.

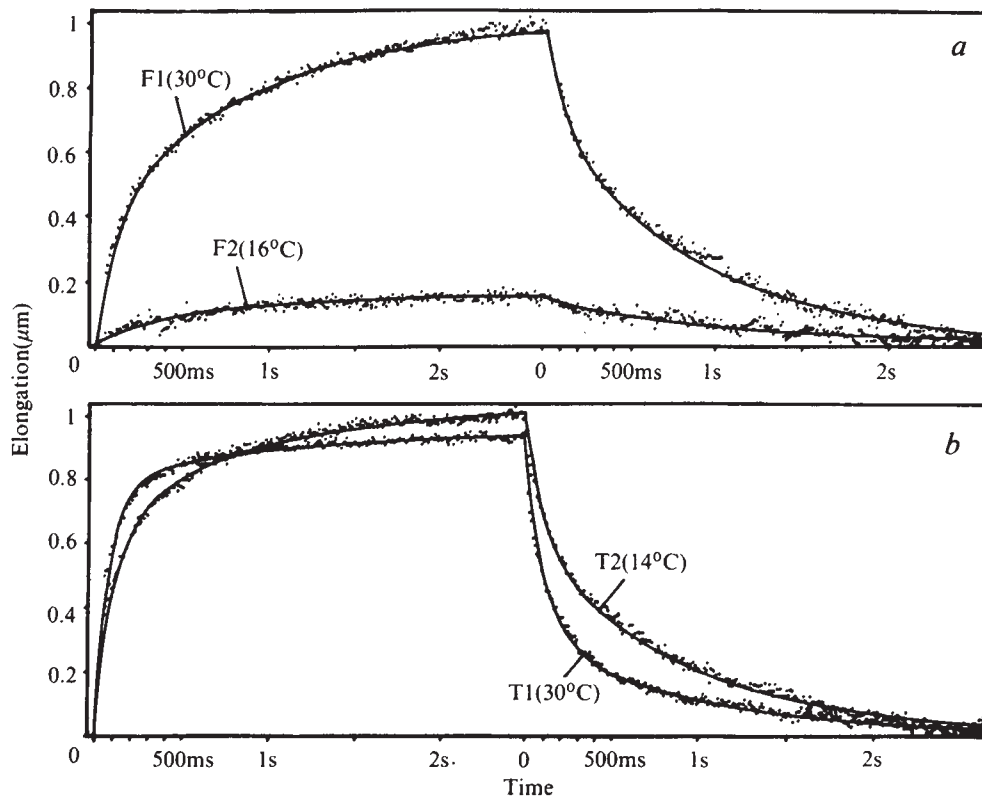
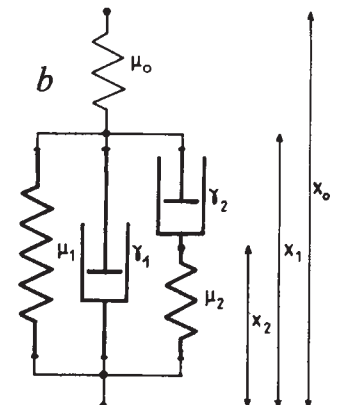
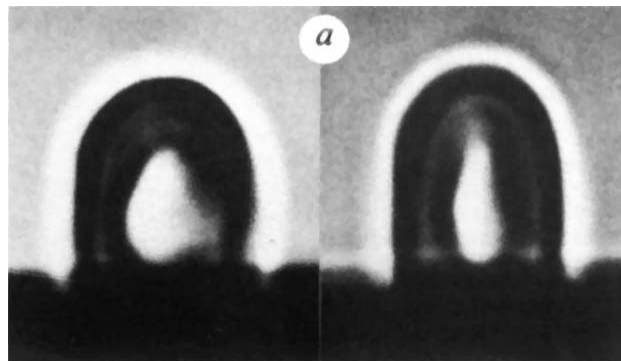


Fig. 2 *a*, Response curves of freshly drawn erythrocytes obtained after application of field jumps of 5 V, $f = 6 \times 10^5$ Hz. The maximum elongation is $A_0 = 1 \mu\text{m}$ at 30°C and $A_0 = 0.21 \mu\text{m}$ at 16°C. The number of repetitions $n = 10$. *b*, Comparison of response curves of trypsinized cells obtained at 30°C and 14°C. The frequency is the same as in *a*, voltage 6 V, repetition number $n = 10$, maximum amplitude $A_0 = 1 \mu\text{m}$ at both temperatures.

Fig. 3 *a*, Photomicrograph of a cell in the presence of a small bias high-frequency field (left) and after increasing the field strength to 10^5 V m^{-1} which leads to a reversible deformation of the cell (right). Note that the cells have a discoid shape in both cases. *b*, Viscoelastic model of a red blood cell membrane consisting of a parallel arrangement of a Maxwell body (right) and a Voigt body (left). The following notations are used: μ_1 , μ_2 , stiffness constants of springs; γ_1 , γ_2 , viscosities of the dash pots; x_1 , x_2 , elongations of springs of Voigt body and Maxwell body, respectively. The spring having the stiffness constant μ_0 has been introduced to account for the fact that the force F_0 is proportional to the elongation x_1 of the blood cell due to the inhomogeneity of the electric field. The model obeys the following differential equations: $\gamma_1 \dot{x}_1 = F_0 - \mu_1 x_1 - \mu_2 x_2$ and $\gamma_2 \dot{x}_2 = \gamma_2 \dot{x}_1 - \mu_2 x_2$.



viscoelasticity is largely determined by the glycocalyx and the spectrin-actin cytoskeleton which are both coupled to the bilayer^{11,12}.

The technique described here enables time-resolved measurements in the linear range of deformation and the application of simple equations of viscoelasticity⁴. This is a major advantage compared with the micropipette method^{5,6,7} and tether formation⁹ or the techniques based on the cellular deformation

in viscometric flows^{12,13}, where non-linear effects have a dominant role. The electric fields are well below the limit of dielectric breakthrough^{15,16} and the forces involved ($F_0 \sim 2 \times 10^{-11} \text{ N}$) are very weak. The field jump technique thus bridges the gap between the non-disturbing methods, based on the frequency analysis of thermally excited membrane undulations¹⁷⁻¹⁹, and the strongly disturbing techniques mentioned above.

Received 24 June; accepted 7 November 1983.

- Pohl, H. A. *Dielectrophoresis* (Cambridge University Press, 1978).
- Holzapfel, C., Vienne, J. & Zimmermann, U. *J. Membrane Biol.* **67**, 13-26 (1982).
- Engelhardt, H. thesis, Munich (1983).
- Fung, Y. C. *Biomechanics* (Springer, New York, 1981).
- Rand, R. P. *Biophys. J.* **4**, 303-316 (1964).
- Evans, E. A. & Skalak, R. *Mechanics and Thermodynamics of Biomembranes* (CRC, Boca Raton, Florida, 1980).
- Hochmuth, R. M., Buxbaum, K. L. & Evans, E. A. *Biophys. J.* **29**, 177-182 (1980).
- Waugh, R. & Evans, E. A. *Biophys. J.* **26**, 115-120 (1979).
- Waugh, R. *Biophys. J.* **38**, 19-27 (1982).

- Kapitzka, H. G. & Sackmann, E. *Biochim. biophys. Acta* **595**, 56-64 (1980).
- Heast, C. W. M. *Biochim. biophys. Acta* **694**, 331-352 (1982).
- Branton, D., Cohen, C. M. & Tyler, I. *Cell* **24**, 24-32 (1981).
- Bessis, M. & Mohandas, M. *Blood Cells* **1**, 307-313 (1975).
- Schmid-Schönbein, H., von Gosen, J., Heinrich, L., Klose, H. J. & Volger, E. *Microvasc. Res.* **6**, 366-376 (1973).
- Zimmermann, U., Scheurich, P., Pwiatt, G. & Benz, R. *Angew. Chem. (Engl. Edn)* **20**, 325-344 (1981).
- Neumann, E., Gerisch, G. & Opatz, K. *Naturwissenschaften*, **67**, 414-415 (1980).
- Servuss, R. M., Harich, W. & Helfrich, W. *Biochim. biophys. Acta* **436**, 900-903 (1976).
- Brochard, F. & Lennon, J. F. *J. de Phys.* **36**, 1035-1047 (1975).
- Fricke, K. & Sackmann, E. *Biochim. biophys. Acta* (in the press).

Effect of IFN- γ on the immune response *in vivo* and on gene expression *in vitro*

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T lymphocytes produce a variety of immunoregulatory molecules including gamma interferon (IFN- γ)¹⁻³ and antigen-specific suppressor and enhancer factors⁴⁻⁷. During our studies of active substances obtained from cloned T-cell lines, we observed that certain fractions administered to mice resulted in enhancement of immune responses. Preliminary characterization of the substance suggested that it could be IFN- γ and we therefore undertook a study of the action of IFN- γ produced by recombinant DNA methodology on immune responses. We found that for several antigens, administration of IFN- γ to mice leads to two- to five-fold enhancement of antibody formation provided that the IFN- γ and antigen are administered together. The effect was dose dependent, giving a maximal response at 500–600 anti-viral units per mouse. Preliminary studies suggest that the macrophage may be the target of IFN- γ action. Addition of IFN- γ to cultures of a macrophage cell line leads to a greater than 10-fold increase in the level of RNA coding for I-region-encoded cell surface molecules.

Various doses of murine IFN- γ , produced by monkey cells transfected with a simian virus 40 (SV40)-based expression vector containing the murine IFN- γ gene, were injected into BDF₁(B6XDBA/2) mice along with the antigen hen egg lysozyme (HEL) or the antigen dinitrophenol-conjugated bovine γ -globulin (DNP-BGG). Administration of IFN- γ affected indirect plaque-forming cell (PFC) responses to both HEL and DNP in a similar dose-dependent manner. A two- to five-fold enhancement of PFC was observed when 500–2,000 units of IFN- γ were administered (Fig. 1a and b). Administration of doses as high as 6,700 or as low as 100 anti-viral units per mouse did not significantly alter the PFC response. Stimulation of the PFC response (direct plaques) using 1,000 units per mouse was also observed with the antigen sheep red blood cells (data not shown). Preparations of IFN- γ derived from independently transfected monkey cells were equally active and showed the same dose-response curve.

The enhancement of the PFC response observed might have been due to recruitment of responding cells to draining lymph nodes, rather than to an actual increase in the number of reactive cells⁸. If the total number of PFC per animal remained unchanged then one would expect that the total antibody secreted per animal would remain constant. Accordingly, serum titres of treated mice were examined. Five mice were injected with 250 units of IFN- γ or phosphate-buffered saline (PBS) on both the day of immunization with the antigen HEL and again on the following day. Individual mice were bled 12 days after immunization and the amount of antibody specific for HEL was determined by radioimmunoassay (monoclonal antibody to HEL was used as a standard). The control group had an average of 1.0 mg ml⁻¹ of anti-HEL antibody whereas the IFN- γ -treated group had an average of 3.4 mg ml⁻¹. The stimulation index of 3.4 agrees with that observed in PFC responses. The range of stimulation indices for individual mice was 1.5–9.2.

The IFN- γ preparations used above contained components of fetal calf serum as well as cellular components secreted by monkey cells. To examine whether the enhancement of the PFC response by IFN- γ preparations was mediated by IFN- γ itself, the preparations were passed over a column containing anti-IFN- γ immunoglobulin. As seen in Table 1, the stimulating

Table 1 Neutralization of enhancing activity of IFN- γ on the PFC response by anti-IFN- γ globulin or treatment at pH 2

Treatment	IFN(U)	PFC per 10 ⁶ cells $\pm$ s.e.	SI	P
Control		1,453 $\pm$ 26	1.00	
None	400	4,448 $\pm$ 214	3.06	<0.001
None	800	7,786 $\pm$ 162	5.36	<0.001
Anti-IFN- γ effluent	(400)	2,325 $\pm$ 114	1.60	NS
Anti-IFN- γ effluent	(800)	1,658 $\pm$ 199	1.15	NS
Anti-IFN- γ eluate	400	5,768 $\pm$ 570	3.97	<0.001
Anti-IFN- γ eluate	800	5,198 $\pm$ 380	3.58	<0.001
Control		666 $\pm$ 21	1.00	
pH 7.3	500	1,761 $\pm$ 179	2.64	<0.005
pH 7.3	1,000	2,535 $\pm$ 209	3.81	<0.001
pH 2.0	(500)	685 $\pm$ 63	1.03	NS
pH 2.0	(1,000)	925 $\pm$ 84	1.39	NS

The monkey cell-derived IFN- γ was passed through a column of Sepharose-4B (Pharmacia) conjugated with anti-IFN- γ globulin (Enzo Biochemical, New York). The material absorbed to the column was eluted with 4 M NaCl. Effluent and eluate were dialysed against 100 volumes of PBS for 24 h. For acid treatment of IFN- γ , the samples were dialysed against either sodium acetate buffer (pH 2.0) or PBS (pH 7.3) for 16 h and were then dialysed against PBS for 24 h. Procedures for immunization, IFN- γ injection and PFC assay were as described in the legend to Fig. 1. HEL was used as an antigen. Units in parentheses indicate the amount of IFN- γ equivalent to those contained in a given volume before treatment. SI, stimulation index; P, probability; NS, not significant.

Table 2 Time dependency of enhancement activity

Injection day	PFC per 10 ⁶ cells $\pm$ s.e.	SI	P
Control	1,348 $\pm$ 158	1.00	
-2, -1	3,693 $\pm$ 466	2.74	<0.01
0, 1	5,946 $\pm$ 767	4.41	<0.005
3, 4	1,949 $\pm$ 456	1.45	NS

Mice were immunized with HEL on day 0 as in Fig. 1 and injected with 250 U of IFN- γ in 0.5 ml of PBS on both days indicated above. The control group was injected with 0.5 ml of PBS on day 0 and day 1. PFC responses were determined as in Fig. 1.

activity was removed by such a treatment but could be subsequently recovered from the column. Similarly, the stimulating activity was sensitive to pH 2.0, a property of IFN- γ (see Table 1). Direct evidence that the enhancement activity was exclusively due to IFN- γ was obtained by using murine IFN- γ isolated from *Escherichia coli* cells expressing a mouse IFN- γ gene. These data are shown in Fig. 1c and d. Two- to four-fold enhancement of PFC responses to HEL and DNP was observed upon administration of IFN- γ at the same doses used earlier. These results strongly indicated that the increase in the PFC response was due to the IFN- γ molecule itself.

To gain some insight into the mechanism of enhancement of antibody formation, we studied the effect of altering the time of administration of IFN- γ relative to antigen challenge. Administration of IFN- γ at the same time as immunization was the most effective (Table 2). Administration of IFN- γ 1 and 2 days before immunization resulted in some stimulation of the anti-HEL response whereas no enhancement was observed if IFN- γ was given on both day 3 and 4 after immunization. This result suggests that perhaps some early step of the immune response such as antigen processing by macrophages could be affected by IFN- γ . IFN- γ , produced by recombinant DNA technology as well as T cells stimulated with mitogens, has been shown to have profound effects on macrophage-dependent cell killing⁹⁻¹¹ and on expression of cell surface molecules associated

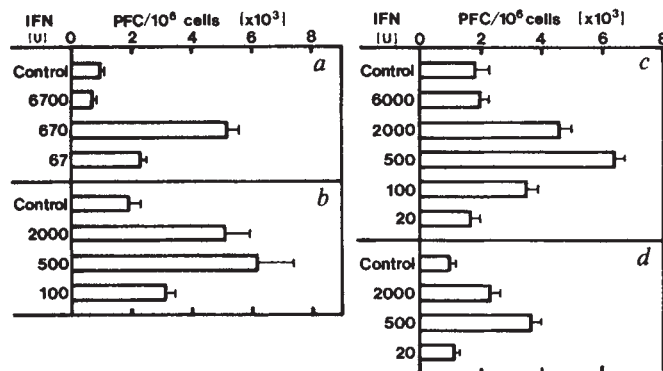


Fig. 1 Effect of IFN- γ on the plaque-forming cell response of mice. Eight- to 12-week-old BDF₁ mice (Jackson Laboratories, Bar Harbor) were immunized with either 100 μ g of HEL (graphs a and c) or DNP-BGG (graphs b and d) in complete Freund's adjuvant intraperitoneally. The IFN- γ preparations, which were supplied by Genentech Inc. (South San Francisco), were culture supernatants (20,000 U ml⁻¹) of the monkey cells transfected with an SV40-based expression vector containing a mouse IFN- γ gene (the approach to its production was basically that which has been reported for the production of cloned human IFN- γ)¹⁶ and a product purified from *E. coli* harboring an expression vector that contained murine IFN- γ cDNA (specific anti-viral activity of 8×10^6 U per mg protein)¹⁷. Left (a and b) and right (c and d) graphs show the results obtained by using the monkey cell-derived and the *E. coli*-derived IFN- γ , respectively. The mice were injected intravenously with various doses of IFN- γ diluted in 0.5 ml of PBS. Injections were on both the day of immunization and the following day. Doses reported are the total amount administered. The control group received 0.5 ml of PBS intravenously. Eight days after immunization a PFC assay was carried out using cells of parathymic lymph nodes for the HEL response or spleen for the DNP response according to a modified method of Jerne¹⁸. HEL- or trinitrophenol-conjugated sheep red blood cells (SRBC) (Scott Laboratories, Fiskeville, Rhode Island) were used as indicator cells. Lyophilized guinea pig serum (Colorado Serum Co.), absorbed on SRBC, was used as a source of complement at a final dilution of 1:40. Rabbit anti-mouse immunoglobulin serum was used at a final dilution of 1:400 to detect IgG PFC. Each bar represents the average PFC response of five mice plus the standard error (s.e.).

with antigen presentation¹²⁻¹⁴. Also it has been reported that recombinant human IFN- γ increases synthesis of HLA-DR molecules on human melanoma cell lines¹⁵. It may be that one of the biological functions of IFN- γ is to convert macrophages from an inactive to an active state, thereby promoting antigen presentation and hence immune responses.

We tested the effect of the bacteria-derived IFN- γ on the expression of *I*-region-encoded antigens in a macrophage cell line, P388D1. As seen in Fig. 2, treatment of cultured cells with doses of IFN- γ of 10–1,000 units ml⁻¹ leads to a profound increase in the level of expression of RNA derived from the *I*-region. The effect was dose dependent and suggests that IFN- γ may act by inducing synthesis of mRNAs that encode proteins involved in antigen processing and presentation.

We have shown that IFN- γ is an immunoregulatory agent as assayed *in vivo* using recombinant DNA-generated preparations. The ability of IFN- γ to enhance the level of induced antibodies was seen with several antigens. The time-dependent nature of the phenomenon suggests that an early step in the immune response is affected. The induction of *I*-region-specific RNA seen in a macrophage cell line suggests that this early step may be antigen processing and/or presentation. Interferon may be a general enhancer of other differentiated functions within the immune system. We are currently investigating whether IFN- γ has demonstrable physiological effects on T and B cells.

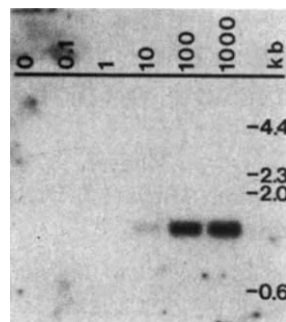


Fig. 2 Increase in the level of *I*-region-encoded RNA due to the addition of IFN- γ to the culture medium of the mouse macrophage cell line P388D1. The DBA/2-derived macrophage cell line P388D1 (ref. 19) was grown in monolayer culture to approximately 30% confluence, whereupon various amounts of *E. coli*-derived murine IFN- γ were added directly to the medium. The cells were then incubated at 37 °C for 48 h and collected. Total cellular RNA was prepared from each cell sample as previously described^{20,21}. 15 μ g of each RNA sample were glyoxalated, electrophoresed in a 1.2% agarose gel and transferred to nitrocellulose as described²². The nitrocellulose filter was then baked for 2 h at 80 °C under vacuum and hybridized (in a buffer containing 50% formamide, 5% SSC and 10% dextran sulphate) at 42 °C for 20 h with an *I*-region (*A_g*) cDNA clone probe (labelled with ³²P via nick-translation) which was kindly provided by Dr Mark Davis, NIH. After multiple washings in 2×SSC at 65 °C the filter was exposed to X-ray film for 48 h using intensifier screens. The resulting autoradiogram is shown. The positions of molecular weight standards run in an adjacent lane (not shown) are indicated. Lane 1, no IFN- γ added; lane 2, IFN- γ added to a final concentration of 0.1 anti-viral units per ml culture medium; lane 3, 1 unit per ml; lane 4, 10 units per ml; lane 5, 100 units per ml; lane 6, 1,000 units per ml. The size of the hybridizing band (1.2 kb) is that expected for *I*-*A_g* mRNA.

The basis of stimulation of *I*-region-derived mRNA is also being studied.

We acknowledge Genentech Inc. for providing the recombinant IFN- γ . We also thank Dr Daniel Capon of Genentech for his help and Audrey Childs for the preparation of this manuscript. This work was supported by NIH grants 1 P01 CA28900 and 5-R01-AI13357 and American Cancer Society grant NP-6M. M.D. is supported by NIH grant 5-F32-CA06873. T.M. is supported by ACS grant PF-2240.

Received 25 July; accepted 28 October 1983.

1. Marcucci, F., Waller, M., Kirchner, H. & Krammer, P. *Nature* **291**, 79–81 (1981).
2. Benjamin, W. R., Steeg, P. S. & Farrar, J. J. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5379–5383 (1982).
3. Le, J., Vilcek, J., Saxinger, C. & Prensly, W. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7857–7861 (1982).
4. Minami, M., Okuda, K., Furusawa, S., Benacerraf, B. & Dorf, M. E. *J. exp. Med.* **154**, 1390–1402 (1981).
5. Takabayashi, K. *et al. J. Immun.* **130**, 2552–2556 (1983).
6. Lonai, P. *et al. J. exp. Med.* **154**, 942–951 (1981).
7. Apte, R. N., Eshhar, Z., Löwy, I., Zinger, H. & Mozes, E. *Eur. J. Immun.* **11**, 931–936 (1981).
8. Korngold, R., Blank, K. J. & Murasko, D. M. *J. Immun.* **130**, 2236–2240 (1983).
9. Meltzer, M. S., Benjamin, W. R. & Farrar, J. J. *J. Immun.* **129**, 2802–2807 (1982).
10. Pace, J. L., Russell, S. W., Torres, B. A., Johnson, H. M. & Gray, P. W. *J. Immun.* **130**, 2011–2013 (1983).
11. Steeg, P. S., Johnson, H. M. & Oppenheim, J. J. *J. Immun.* **129**, 2402–2406 (1982).
12. McNicholas, J. M., King, D. P. & Jones, P. P. *J. Immun.* **130**, 449–456 (1983).
13. King, D. P. & Jones, P. P. *J. Immun.* **131**, 315–318 (1983).
14. Wong, G. H., Clark-Lewis, L., McKimm-Breschkin, J. L., Harris, A. W. & Schrader, J. W. *J. Immun.* **131**, 788–792 (1983).
15. Basham, T. Y. & Merigan, T. C. *J. Immun.* **130**, 1492–1494 (1983).
16. Gray, P. W. *et al. Nature* **295**, 503–508 (1982).
17. Gray, P. W. & Goeddel, D. V. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5842–5846 (1983).
18. Mishell, B. B., Shiigi, S. M. & Mishell, R. I. in *Selected Methods in Cellular Immunology* (eds Mishell, B. B. & Shiigi, S. M.) 72–77 (Freeman, San Francisco, 1980).
19. Koren, H. S., Handwerker, B. S. & Wunderlich, J. R. *J. Immun.* **114**, 894–897 (1975).
20. Glisin, V., Crkvenjakov, R. & Byus, C. *Biochemistry* **13**, 2633–2637 (1974).
21. Ullrich, A. *et al. Science* **196**, 1313–1319 (1977).
22. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).

Surface dynamics of the integral membrane protein bacteriorhodopsin

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Recently, there has been great interest in determining the three-dimensional structures of membrane proteins, particularly bacteriorhodopsin, for which a variety of possible folding arrangements have been suggested¹⁻⁴. In this paper we present nuclear magnetic resonance (NMR) spectra⁵⁻⁸ of deuterated bacteriorhodopsin, and use the data to help interpret the various suggested bacteriorhodopsin folding patterns. The results strongly indicate that (1) a membrane surface (± 1 residue) may be defined by NMR in bacteriorhodopsin; (2) all amino acids inside the surface are essentially crystalline; (3) all amino acids outside the surface (surface residues) in bacteriorhodopsin are highly mobile on the time scale of the ^2H NMR experiments; (4) NMR data may be used to help evaluate the various structural models that have been proposed; (5) aggregation of purple membrane sheets may lead to an immobilization of the surface residues.

In Fig. 1 A-I, we show ^2H NMR spectra of a variety of ^2H -labelled bacteriorhodopsin samples. In all cases, the spectrum observed consists of a sharp central component and a broad powder pattern. The central component arises, we believe, from surface residues in bacteriorhodopsin. Observation of this component requires acquisition of at least 2,000-4,000 data points, Fig. 1, J-M, to avoid truncation effects and its 'disappearance'. The component can then be quantified by means of computer simulation.

The central components arise from deuterons that reorient over a wide angular range in a time several-fold less than the inverse of the frequency of the rigid lattice quadrupolar splitting, that is, $\tau_c \ll 10^{-5}$ s. In principle, the deuterium could be in very mobile surface residues, in isotropically tumbling membrane fragments, or perhaps in residual HO^2H in our sample. We rule out the possibility that residual HO^2H is a significant contributor to the central component because additional exchanges of the samples with ^2H -depleted water do not reduce the intensity of the central component, and the absolute intensity of a corresponding unlabelled membrane sample, exchanged with ^2H -depleted water, is negligible. All samples (with different labelling of the amino acids by ^2H) contain essentially the same protein/water ratios, having been exchanged three times with deuterium-depleted water by centrifugation (for 14 h at 130,000g) followed by resuspension in deuterium-depleted water (200 mg membrane per 5 ml water), but the intensity of the central component varied widely. Even lyophilization and resuspension in ^2H -depleted water does not change the intensity of the isotropic components (data not shown).

We rule out the possibility that the central components arise from isotropically tumbling membrane fragments since then the isotropic/rigid ratios should be the same for each labelled amino acid, which is not the case. Instead, there is a strong correlation between the position of the residue within the purple membrane postulated by the various experimental and theoretical models¹⁻⁴, and the intensity of the central component resonances of Fig. 1 A-I. We thus ascribe at least 90-95% of the intensity of the central component to surface residues undergoing fast isotropic motion.

In Fig. 2 we show the folding pattern of bacteriorhodopsin in the purple membrane of *Halobacterium halobium* postulated recently by Engelman *et al.*⁴. To interpret our ^2H NMR results, we define a membrane surface, designated B in Fig. 2. Residues to the outside of line B are surface residues, which we assert will be mobile on the ^2H NMR timescale (giving the central

'isotropic' components, Fig. 1, A-I), while residues inside of line B will be rigid, or crystalline, and lead to the broad powder-pattern line shapes of Fig. 1. Similarly, we define two alternative membrane surfaces, A and C, which are two residues outside or inside of line B respectively. The earlier models of Engelman *et al.*¹, Ovchinnikov² and Khorana³ may be treated similarly, to define various possible membrane surface planes.

We show in Fig. 3 A-D, the correlations we have obtained, for a selection of deuterium-labelled amino-acids, between the percentage of the residues located on the surface of bacteriorhodopsin (for a particular model, refs 1-4), and the percentage of the total ^2H signal intensity of the isotropic component (mobile, surface residues) obtained from simulations of the spectra of Fig. 1 (and data at 37 °C for Phe, Tyr and Arg, not shown). There is a significant correlation for all models, Fig. 3

Table 1 Correlation between the number of surface residues predicted for various theoretical models of bacteriorhodopsin structure and the experimental ^2H NMR results

Model	Slope (m)	Intercept (c)	Correlation coefficient (ρ)
Ideal*	1.00	0.00	1.00
Wertz-Scheraga†	0.98	17.5	0.61
Engelman, 1980‡	1.12	-3.1	0.80
+1§	0.89	-3.3	0.81
+2	0.56	-1.2	0.69
-1	1.24	1.7	0.86
-2	1.12	12.1	0.70
Ovchinnikov, 1982	1.08	2.0	0.87
+1	0.90	-1.1	0.79
+2	0.64	-1.6	0.64
-1	1.15	5.3	0.85
-2	1.09	15.2	0.68
Khorana, 1982¶	1.10	-0.2	0.85
+1	0.76	0.7	0.77
+2	0.60	0.2	0.64
-1	1.29	3.3	0.85
-2	1.15	13.9	0.63
Engelman, 1982#	0.93	3.0	0.90
+1	0.77	1.4	0.76
+2**	0.69	-2.1	0.69
-1	1.20	4.6	0.82
-2††	1.25	10.8	0.68

Results were calculated assuming a 1:1 correspondence between the % surface residues for a given model (S , %) and the intensity (I , %) of the central, isotropic ^2H NMR component as $S(\%) = m(I, \%) + c$.

* For perfect agreement, $m = 1.00$, $c = 0.00$ and $\rho = 1.00$, where ρ is the correlation coefficient, m is the slope and c is the intercept, as defined above. † Ref. 9. ‡ Ref. 1. § Surface defined as 1 and 2 residues outside (+) and 1 and 2 residues inside (-) from published model, respectively. || Ref. 2. ¶ Ref. 3. # Ref. 4, line B in Fig. 2. ** Line A in Fig. 2. †† Line C in Fig. 2.

and Table 1. The earliest model of Engelman *et al.* (ref. 1, Fig. 3A) has the poorest agreement with the ^2H NMR results. We predict a theoretical slope (m) of 1.0, for a 1:1 correspondence between percent surface residues and percent isotropic component, while the model gives $m = 1.12$ with a correlation coefficient $\rho = 0.80$. The later models of Ovchinnikov (Fig. 3B), Khorana (Fig. 3C) and Engelman (Fig. 3D) all give considerably better agreement (Table 1). Each part of Fig. 3 includes the perfect agreement, theoretical line ($m = 1$, $c = 0$).

We have also examined each of the published bacteriorhodopsin models using changes in surface definition of ± 1 , and ± 2 residues. The results indicate that the originally proposed surfaces gave the best fit (Table 1). As an example, we show in Fig. 3E typical results for the Engelman/82 model⁴. For a +2 and a -2 residue change in surface definition the correlation with the NMR results is much poorer (+2, $m = 0.69$, $c = -2.1$, $\rho = 0.69$; -2, $m = 1.25$, $c = 10.8$, $\rho = 0.68$).

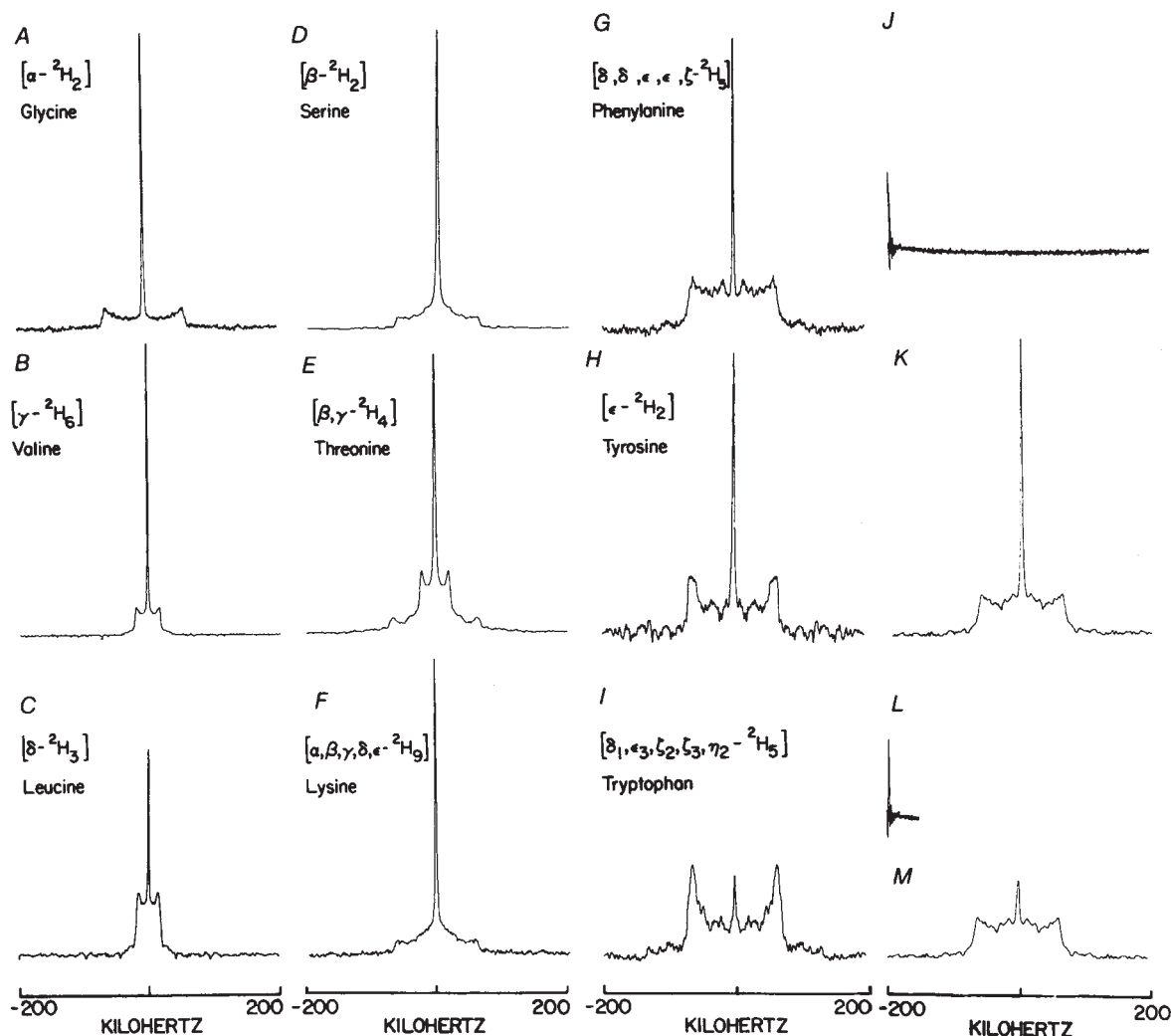


Fig. 1 Experimental ^2H NMR spectra (at 55.3 MHz and 4°C) of selectively ^2H -labelled membranes, showing intensity of the central (surface) component, and truncation effects. A–I, ^2H -labelled amino acid, as indicated. J–M, $[\text{H}_5]$ phenylalanine-labelled bacteriorhodopsin in the time and frequency domains. J, K, 4,096 data points, L, M, 512 data points. Note that inadequate digitization causes a loss of central-component intensity. The spectra were obtained on a home-built instrument which consists of a 3.5-inch bore 8.45 Tesla superconducting solenoid (Oxford Instruments), a Nicolet 1180 computer interfaced to a Nicolet NIC-2090 50-ns transient recorder system (Nicolet), and a variety of digital and radiofrequency electronics¹⁰. Spectra were recorded for a 800- μl sample, with a quadrupole-echo pulse sequence¹¹ using a 90° pulse width of $\sim 3\ \mu\text{s}$. The spectrometer was prepared for data acquisition as described previously¹². DL[β - $^2\text{H}_4$]serine was synthesized from ethyl acetamidocyano-acetate by reduction with sodium borodeuteride, followed by hydrolysis with strong acid¹³ and recrystallization from aqueous ethanol. DL[β , γ - $^2\text{H}_4$]threonine ($\sim 75\%$ in the *threo* form) was synthesized by condensing [$^2\text{H}_4$]acetaldehyde (Merck, Sharpe and Dohme) with *bis*-glycine copper (II)¹⁴. Copper was removed on a column of Dowex 50W-X8 (Bio-Rad) with 5M NH_4OH as the eluant. After removal of the solvent under reduced pressure, the DL[β , γ - $^2\text{H}_4$]threonine was recrystallized from methanol/water (4: 1, v/v). [α - $^2\text{H}_2$]glycine, L[δ - $^2\text{H}_3$]leucine, and DL[α , β , γ , δ , ϵ - $^2\text{H}_9$]lysine were purchased from Merck, Sharpe and Dohme, and L[α , β , γ , δ , ϵ - $^2\text{H}_{10}$]isoleucine was from Cambridge Isotopes. DL[γ - $^2\text{H}_6$]valine, L[δ_1 , δ_2 , ϵ_1 , ϵ_2 , ζ - $^2\text{H}_5$]phenylalanine, L[ϵ_1 , ϵ_2 - $^2\text{H}_2$]tyrosine and L[δ_1 , δ_2 , ϵ_1 , ϵ_2 , ζ - $^2\text{H}_5$]tryptophan were prepared as described previously (refs 15, 16 and references cited therein). *Halobacterium halobium* R₁ (the kind gift of Professor T. Ebrey) was grown and harvested as described previously^{15,16}. Deuterated amino acids were incorporated into a defined medium at the following concentrations: [$^2\text{H}_2$]Gly, 0.6 g per 10l; DL[γ - $^2\text{H}_6$]Val, 10.0 g per 10l; L[δ - $^2\text{H}_3$]Leu, 0.2 g per 5l; L[α , β , γ , δ , ϵ - $^2\text{H}_{10}$]Ileu, 0.2 g per 5l; DL[β - $^2\text{H}_2$]Ser, 3.0 g per 10l; DL[β , γ - $^2\text{H}_4$]Thr, 5.0 g per 10l; L[α , β , γ , δ , ϵ - $^2\text{H}_9$]Lys, 0.2 g per 1l; L[δ_1 , δ_2 , ϵ_1 , ϵ_2 , ζ - $^2\text{H}_5$]Phe, 1.3 g per 10l; L[ϵ_1 , ϵ_2 - $^2\text{H}_2$]Tyr, 2.5 g per 10l; L[δ_1 , δ_2 , ϵ_1 , ϵ_2 , ζ - $^2\text{H}_5$]Trp, 5.0 g per 10l. We have assumed that only L-amino acids were incorporated when DL mixtures were added to the growth medium. Purple membranes were isolated essentially by the method of Becher and Cassim¹⁷, as described previously^{15,16}. Membranes were exchanged three times with ^2H -depleted water (Aldrich) prior to NMR spectroscopy, by equilibrating in the H_2O (unbuffered and salt free) for 1 h at 23°C , followed by centrifugation at 130,000g for ~ 14 h. Amino acid incorporation was checked in radiotracer experiments with 50 μCi of uniformly [^{14}C]labelled amino acids per 500ml growth medium as described previously^{15,16}. Val, Leu, Ileu, Thr, Lys, Phe, Tyr and Trp showed essentially no ($\leq 5\%$) breakdown and reincorporation into other amino acids. However, only 52% of the ^{14}C -Ser counts in the purple membrane hydrolysate were found to be associated with that amino acid, the other radioactivity being associated with glycine (16%), alanine (12%) and tryptophan (9%). Similarly, only 81% of the ^{14}C -Gly counts were associated with glycine, the other radioactivity being incorporated into tyrosine (12%) and serine (6%). The amounts of endogenous amino-acid synthesis were not determined.

We also show in Fig. 3F the correlation between the per cent residues on the surface of a typical globular protein (the Wertz-Scheraga scale)⁸, and the per cent isotropic component. This shows an even poorer agreement ($m = 0.98$, $c = 17.5$, $\rho = 0.61$), illustrating we believe the large differences between the membrane protein bacteriorhodopsin and a typical globular protein.

Finally, we note that the ^2H NMR spectra of highly aggregated purple membrane sheets (obtained by mild tryptic cleavage of the C-terminal residues) do not exhibit the intense central components characteristic of mobile surface residues. For example, in the case of [γ - $^2\text{H}_6$]valine, the central component contributes 35% to the intensity of the untreated membrane

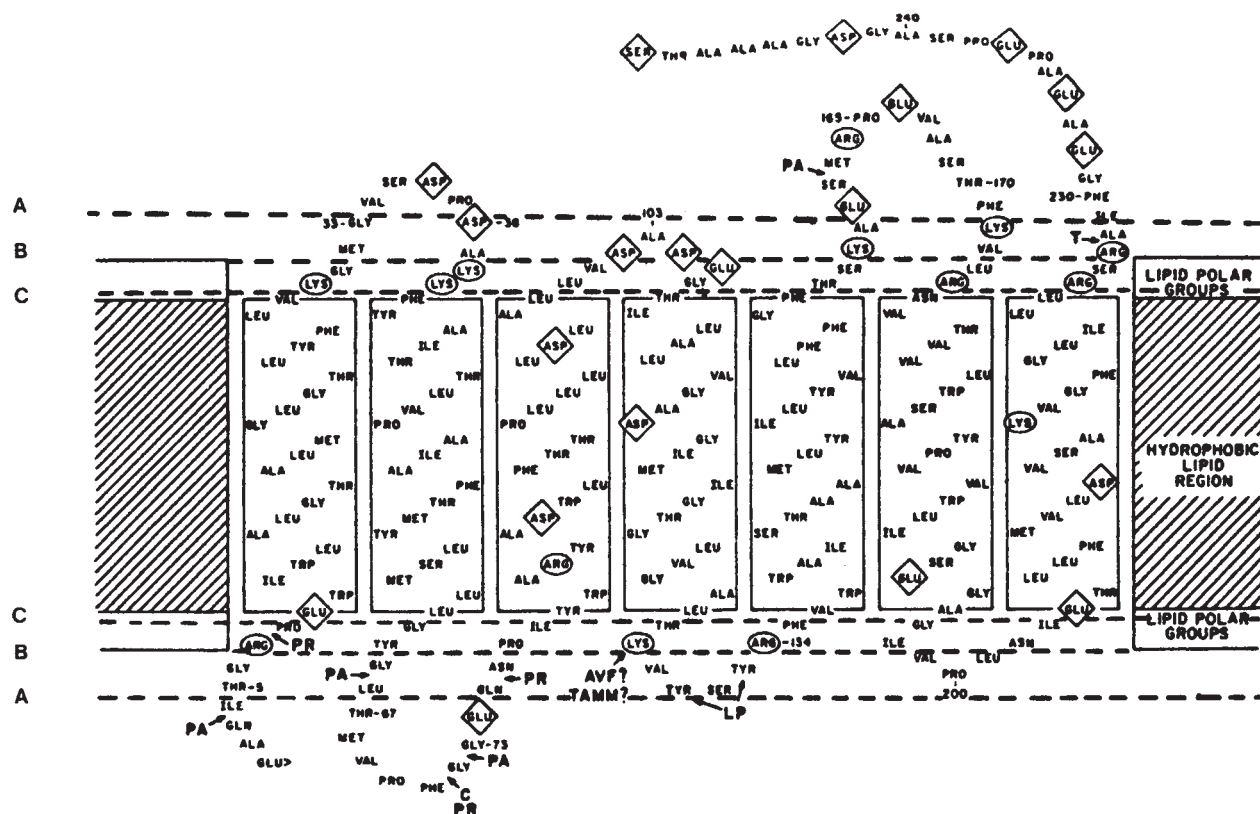
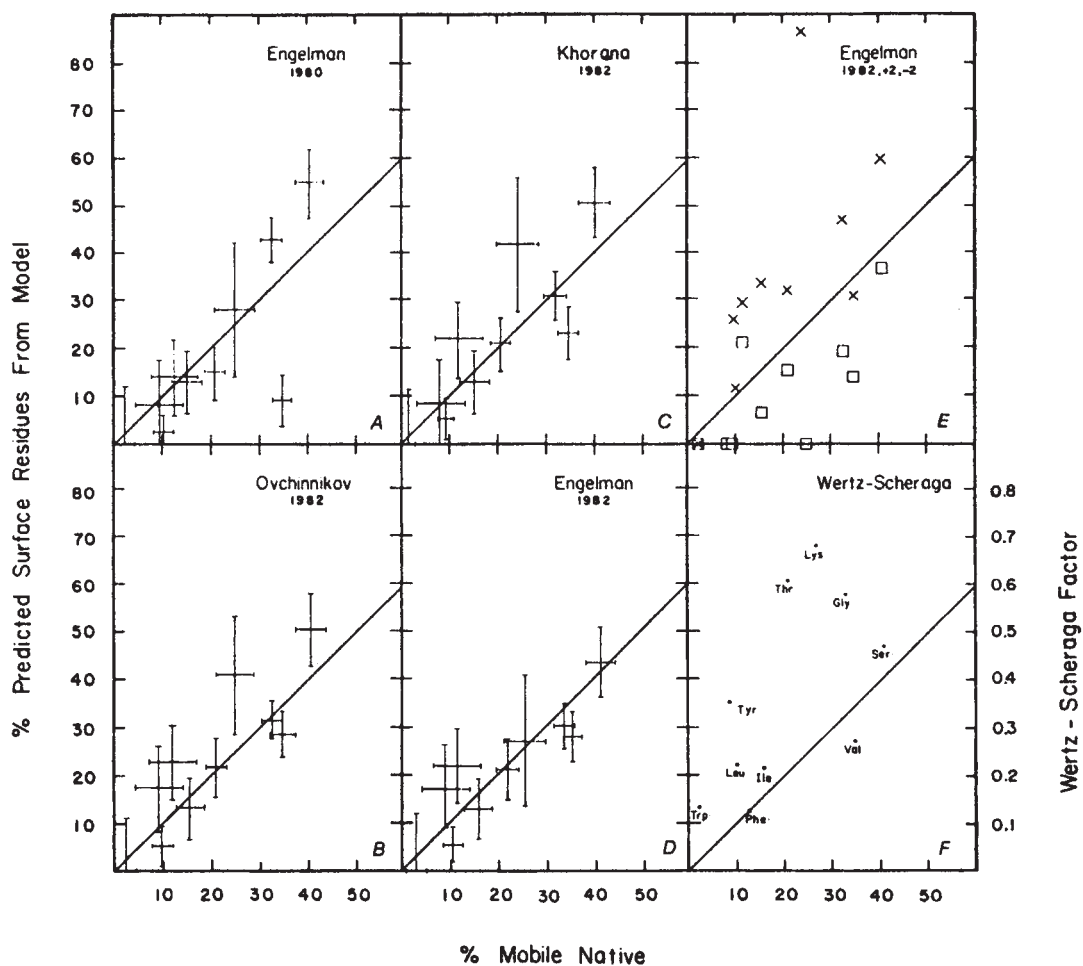


Fig. 2 Proposed model of secondary structure of bacteriorhodopsin (ref. 4) showing various definitions of the membrane surfaces (A, B and C).

Fig. 3 Correlations between per cent surface residues for various theoretical models of bacteriorhodopsin structure, and per cent isotropic component in the ^2H NMR spectrum (Fig. 2). A, Engelman, 1980 model. B, Ovchinnikov, 1982 model². C, Khorana, 1982 model³. D, Engelman, 1982 model⁴. E, Engelman, 1982 model using as surface definitions lines A (+2, $\square$) and C (-2, $\times$) from Fig. 2. F, Correlations with the Wertz-Scheraga index⁹. The vertical error bars are percentage errors for an error of $\pm$ one residue. The horizontal error bars represent an estimate of the experimental error in the central component intensity determination. The straight lines in each part are the theoretical relationship ($m=1$, $c=0$).



spectrum but only 7% to the intensity of the spectrum of the membrane after digestion, even though no valines are removed (unpublished results). This suggests that the motional states of membrane surface residues are highly sensitive to the nature of the surroundings, and such dynamic interactions could have a role in processes such as cell-cell recognition.

This work was supported in part by the US NIH and the US NSF. E.O. is a PHS Research Career Development Awardee.

Received 21 June; accepted 25 October 1983.

- Engelman, D. M., Henderson, R., McLachlan, A. D. & Wallace, B. A. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2023–2027 (1980).
- Ovchinnikov, Y. A. *FEBS Lett.* **148**, 179–191 (1982).
- Huang, K.-S., Radhakrishnan, R., Bayley, H. & Khorana, H. G. *J. biol. Chem.* **257**, 13616–13623 (1982).
- Engelman, D. M., Goldman, A. & Steitz, T. A. *Meth. Enzym.* **88**, 81–88 (1982).
- Kang, S. Y., Gutowsky, H. S. & Oldfield, E. *Biochemistry* **18**, 3268–3271 (1979).
- Barnes, R. G. in *Advances in Nuclear Quadrupole Resonance* (ed. Smith, J. A. S.) Vol. 1, 335–355 (Pergamon, New York, 1974).
- Oldfield, E., Meadows, M., Rice, D. & Jacobs, R. *Biochemistry* **17**, 2727–2740 (1978).
- Oldfield, E. *et al. Biochem. Soc. Symp.* **46**, 155–181 (1980).
- Wertz, D. H. & Scheraga, H. A. *Macromolecules* **11**, 9–15 (1978).
- Oldfield, E. & Meadows, M. *J. mag. Res.* **31**, 327–335 (1978).
- Davis, J. H., Jeffrey, K. R., Bloom, M., Valic, M. I. & Higgs, T. P. *Chem. phys. Lett.* **42**, 390–394 (1976).
- Rothgeb, T. M. & Oldfield, E. *J. biol. Chem.* **256**, 1432–1446 (1981).
- Berlinquet, L. *Can. J. Chem.* **33**, 1119–1129 (1955).
- Sato, M., Okawa, K. & Akabori, S. *Bull. Chem. Soc. Japan* **30**, 937–938 (1957).
- Kinsey, R. A., Kintanar, A. & Oldfield, E. *J. biol. Chem.* **256**, 9028–9036 (1981).
- Kinsey, R. A. *et al. J. biol. Chem.* **256**, 4146–4149 (1981).
- Becher, R. M. & Cassim, J. Y. *Prep. Biochem.* **5**, 161–178 (1975).

Preferential integration of yeast transposable element Ty into a promoter region

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Mobile genetic elements have been identified in several eukaryotic organisms and some classes have been found to share common structural features with the proviral forms of animal retroviruses^{1–7}. The representatives of this class of mobile elements in the yeast *Saccharomyces cerevisiae* are called Ty elements⁸, which could be a useful model system for studying the transposition of retrovirus-like elements. Here we have attempted to answer two questions often raised in discussions of the biological importance of transposition: what is the frequency of spontaneous Ty transposition, and are there certain chromosomal regions into which Ty elements preferentially integrate? We chose the *LYS2* gene to investigate these questions because it allows direct selection of both mutants and revertants⁹. We have found that 2% of spontaneous *lys2* mutants are caused by Ty transposition with a preferential integration into the transcription initiation region.

303 mutant strains with no (*lys2*) or significantly reduced (*lys2aau*) expression of the *LYS2* gene were isolated from two haploid *S. cerevisiae* strains as described in Fig. 1 legend. Both strains carried about 35 Ty elements^{2,8}. Total DNA from groups of five or six of these mutants was cleaved with the restriction nucleases *XhoI* or *XhoI* plus *BglII*. These minipools were screened by DNA hybridizations¹⁰ for size alterations in the wild-type *LYS2* restriction fragments using the cloned *LYS2* gene¹¹ as probe (Fig. 1a). Non-wild-type hybridization signals were observed within five minipools, and examples are documented in Fig. 1b. Rescreening of the individual strains in the five minipools identified in each case one mutant strain with altered *LYS2*-specific restriction fragments; three *lys2* mutants (*lys2-4*, *lys2-8*, *lys2-19*) and two *lys2aau* mutants (*lys2-17*, *lys2-655*). Further analysis with *BamHI* cleaved DNAs revealed

in all cases an approximately 6-kilobase(kb) insertion as documented in Fig. 1c.

To identify the inserted sequences, we cloned and analysed at first the *lys2-655* allele. After determining the sizes of restriction fragments which would cover part of the unaltered *LYS2* gene and part of the inserted DNA, we chose a 5.6-kb *BamHI-SalI* fragment for cloning into pBR322. The restriction map of this clone (pCH655) is shown in Fig. 2a, together with the restriction maps of Ty1 and Ty2, the two known types of Ty elements^{12,13}. A comparison clearly identifies the insertion in *lys2-655* as a Ty1 element. Furthermore, hybridization experiments against *EcoRI*-cleaved S288C DNA using pCH655 as probe revealed a typical Ty1 pattern⁸ (data not shown). Since the *lys2-655* mutant could grow without lysine (*lys2aau* phenotype) it is not surprising that the Ty1 had inserted in this mutant into the promoter region, as indicated in Fig. 2a, and not in the transcribed portion of the *LYS2* gene. The actual distance between the Ty1 integration site and the start of the *LYS2* coding region is 107 base pairs (bp) as determined by DNA sequence analysis (D. Pridmore and P.P., unpublished).

The expression of the *lys2-655* allele is only 20% of the wild-type level (H.E., M. Winston, J. K. Bhattacharjee and P.P., manuscript in preparation). We assume that the reduced transcription is still directed by *LYS2* promoter elements and not by δ promoter sequences in the Ty ends³, as δ promoted transcription would proceed away from the *LYS2* gene as judged from the Ty1 orientation in this insertion mutant.

The insertions in the other four mutants were identified by an extensive hybridization analysis using five restriction nucleases as Ty1 elements in the three *lys2* mutants *lys2-4*, *lys2-8*, *lys2-19* and as a Ty2 element in *lys2-17*, the second *lys2aau* mutant. Examples are documented in Fig. 2b and c for the *XhoI* and *XhoI-BglII* hybridization spectra, respectively. These five cases of Ty insertions were found among 303 spontaneous *lys2* and *lys2aau* mutants. Such mutants occur with a frequency of 5×10^{-6} in haploid *S. cerevisiae* (ref. 9 and Fig. 1 legend). The spontaneous frequency of Ty transposition in these strains, which carry around 35 Ty elements^{2,8}, is therefore approximately 10^{-7} .

XhoI cleaves most Ty elements in their terminal δ sequences^{12,13} and cleaves once within the *LYS2* gene¹¹. *XhoI*-cleaved DNA from all five mutant strains showed a strong signal at around 3 kb (Fig. 2b). This indicates a clustering of the Ty insertion sites 3 kb upstream from the *LYS2 XhoI* site, a region which coincides with the *LYS2* promoter. A more precise localization of the Ty insertion sites was obtained with *XhoI-BglII*-co-digested DNA using the 650-bp *BglII-EcoRI* fragment as probe (see Fig. 1a), which contains the *LYS2* promoter. This probe detects only short restriction fragments (Fig. 2c). The sizes of these fragments minus the known distance between the Ty end and the $\delta XhoI$ site revealed the integration sites of the respective Ty elements. The result is presented in Fig. 3. Four of the five Ty insertions map within 40–110 bp in front of the *LYS2* translation start and one Ty maps 220 bp after this start.

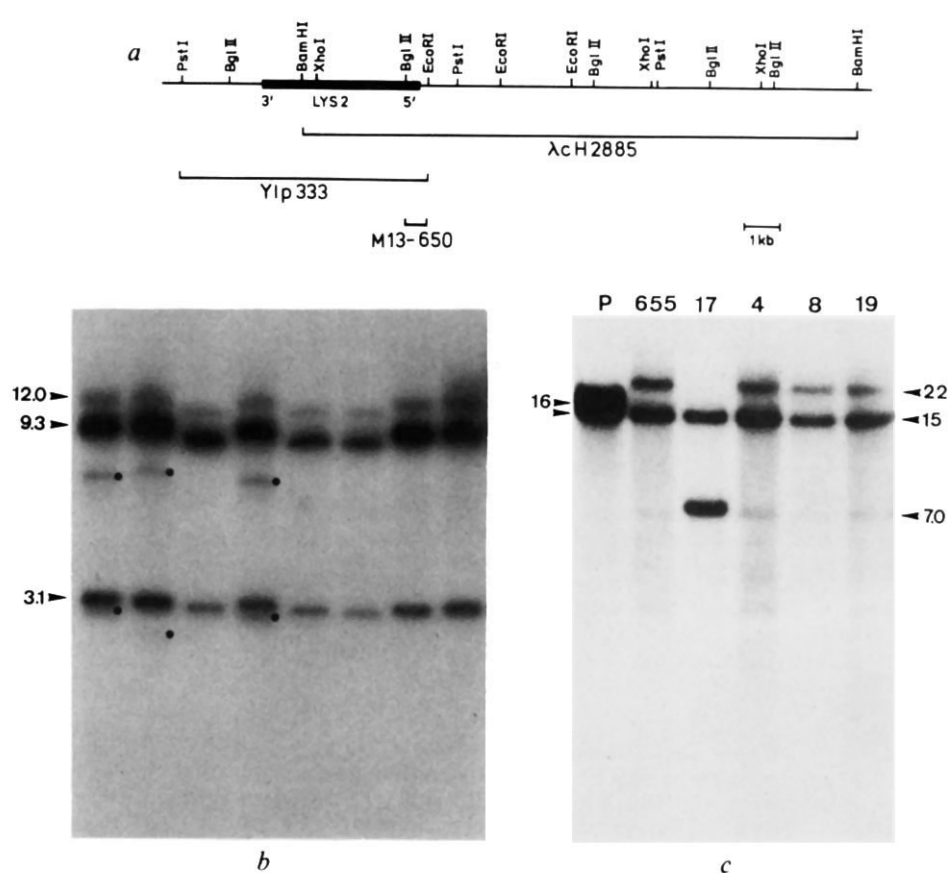
This clustering around the start of the 4,000-bp long *LYS2* gene is remarkable, since any Ty insertion within the coding part of the gene would have been detected as a *lys2* mutant in the selection procedure. As the α -amino adipate-based selection for *lys2* mutants allows the isolation of a great variety of *lys2* mutants, including nonsense and missense mutations¹⁴, its influence on the nonrandom distribution of Ty elements is highly improbable. In addition, insertion of DNA into the *LYS2* gene is not *a priori* impossible; for example, when *S. cerevisiae* cells are transformed with linear DNA harbouring *LYS2* sequences at both ends, an efficient insertion into the *LYS2* coding region is observed¹¹ resulting in a *lys2*⁺ phenotype.

The target preference of Ty elements is therefore best explained as an intrinsic property of the elements, which enables Ty elements to discriminate between favourable targets and other less 'attractive' DNA sequences. This could be based on the ability of the Ty transposition machinery to recognize differ-

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Fig. 1 Screening of *lys2* mutants for DNA rearrangements. **a**, Restriction map of the *LYS2* gene region in chromosome II from *S. cerevisiae*¹¹ and origin of *LYS2* probes. λ CH2885 carries the 16-kb *Bam*HI fragment cloned into λ 1059 and YIp333 carries the 6.9-kb *Pst*I–*Eco*RI fragment cloned into pBR322¹¹. M13–650 carries the 650-bp *Bgl*II–*Eco*RI fragment of the *LYS2* promoter region cloned into the M13 vector mp8³³. **b**, Hybridization signals of *Xho*I restriction digests obtained with pools of mutant DNAs. Cleaved DNA was separated in 0.5% agarose and probed with λ CH2885. The 9.3- and 3.1-kb signals correspond to internal *Xho*I fragments of the probe (see **a**); the 12-kb signal represents the *Xho*I fragment extending to the right of the probe. The *Xho*I fragment extending to the left of the probe is ~20 kb and was not transferred in most cases. New signals pointing to DNA rearrangements are marked in three of the eight lanes. Each lane contained a DNA mixture from six spontaneous *lys2* mutants obtained from strain V2. **c**, *LYS2*-specific *Bam*HI restriction fragments in parental and mutant strains. DNA of the parental strain S288C and the mutants *lys2*–655, *lys2*–17, *lys2*–4, *lys2*–8 and *lys2*–19 (from left to right) was cleaved with *Bam*HI,



separated in 0.5% agarose and hybridized with YIp333. The parental DNA (lane P) exhibits the two expected signals of 15 and 16 kb, which correspond to the sequences extending to the left (3') and right (5') of the cleaved *Bam*HI site in the *LYS2* gene, respectively (see **a**). All mutant DNAs have lost the 5'-specific 16-kb signal and four show a 22-kb signal instead, indicating a 6-kb insertion. DNA from the mutant *lys2*–17 also carries a 6-kb insertion in the 16-kb fragment but one with a *Bam*HI site, leading to a 7- and a 15-kb signal. The latter one co-migrates in this case with the unchanged 15-kb fragment from the 3' side, but its existence could be clearly demonstrated using λ CH2885 as probe (not shown).

Methods: The selection for *lys2* mutants is based on the use of α -amino adipate as sole nitrogen source as described by Chattoo *et al.*⁹. 95% or more of *S. cerevisiae* cells which grow on α -amino adipate plus lysine are *lys2* mutants; among them are some with residual activity, called *lys2aau* mutants. We used the two haploid strains V2 (a *trp1 his4*) and S288C (α *gal2 mal*) as source for mutants. The frequency of spontaneous *lys2* plus *lys2aau* mutants was in both cases 5×10^{-6} per cell, which is in agreement with previous work⁹. Cells from 60 single colonies of strain V2 were grown to stationary phase in full medium and 5×10^7 cells each were plated on α -amino adipate plus lysine. From each plate we collected only one *lys2* and one *lys2aau* mutant in order to be sure to analyse independent mutation events. In total we collected 54 *lys2* and 58 *lys2aau* mutants. A second set of mutants was derived from strain S288C. Cells were grown this time in minimal medium (lacking lysine) to the end of the logarithmic phase and plated at high densities on selective medium. After an incubation at 30 °C for 5 days, 196 mutant colonies were obtained. 176 of these mutants complemented a *lys5* tester strain but not a *lys2* tester strain and were classified as *lys2*. 15 of the mutants grew on selective medium but also on medium lacking lysine and complemented *lys2* and *lys5* mutations; they were classified as *lys2aau*. The remaining 5 mutants were *lys5*. DNAs were isolated as described previously¹¹. Hybridization of the cleaved DNA after transfer to nitrocellulose was performed according to Southern¹⁰ with the modifications introduced by Smith and Summers³⁴. Nick translation³⁵ was used to label the hybridization probes. The lengths of the restriction fragments were determined from a comparison with co-migrating λ HindIII fragments³⁶.

ences in the chromatin structure of regulatory and coding sequences resulting in a higher affinity for opened regions. DNA sequence characteristics which would lead to such opened regions have not yet been elucidated. It has been noted previously that target regions for Ty transposition contain 60–80% A+T base pairs^{15,16,20} in a genome with an average A+T content of 60%. The DNA sequence at the beginning of the *LYS2* gene shows one A+T-rich region (72%) centred around 70 bp in front of the translation start and another A+T-rich region (80%) centred around 240 bp after the start (D. Pridmore and P.P., unpublished). These locations coincide with the Ty integration sites.

Our data do not allow any conclusion as to whether the 3'-flanking regions of genes are also Ty landing sites, as such insertions would probably not be detected in the selection system used. In this context it is also important whether our observations made with Ty insertions at the *LYS2* locus are specific for that gene, or if similar events also occur at other loci in *S. cerevisiae*. Ty insertions have been observed in front of the *HIS4* (refs 16–18), *ADH2* (refs 19, 20), *CYC7* (refs 21, 22), *CAR1* (ref.

23) and *HIS3* (ref. 24) genes. The selection system used to recover these mutants would not have allowed the detection of Ty insertions into coding regions, except for *HIS4*, where two Ty insertions in front of the gene were characterized^{16–18}. A conclusion as to the target specificity would have been difficult, if not impossible, from these studies. The nonbiased *lys2* selection system with its potential for isolating many Ty insertion mutants therefore allows a thorough analysis of Ty transposition in yeast.

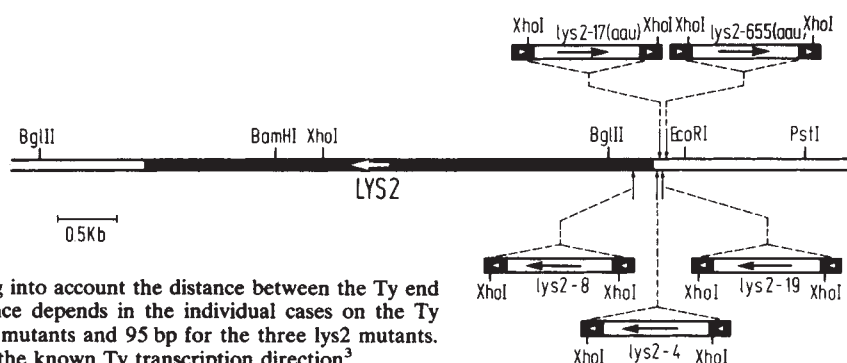
Several laboratories have recently found Ty elements or δ sequences close to the 5' end of several tRNA genes (summarized in ref. 25). If δ sequences and tRNA genes were randomly distributed, they should be about 20–30 kb apart in the yeast genome. The close association found is therefore remarkable and may again reflect the attractiveness of regions 5' to transcribed sequences as Ty landing sites.

Retrovirus-like mobile elements in higher eukaryotes were also frequently detected around promoter regions^{26–32} and further studies may confirm a preference for such regions as integration sites. Thus insertions of these elements would frequently

Fig. 2 Identification and mapping of Ty elements at the *LYS2* locus. **a**, Restriction map around the *LYS2*-Ty junction of the mutant *lys2*-655 as determined from the cloned 5.6-kb *Bam*HI-*Sal*I segment. The known restriction maps of Ty1 and Ty2^{12,13}, which differ by a 3.5-kb substitution, are also shown. B, *Bam*HI; X, *Xho*I; Bg, *Bgl*II; P, *Pst*I; S, *Sal*I; E, *Eco*RI; H, *Hind*III; C, *Cla*I. The thin black line represents transcribed *LYS2* sequences¹¹. Black boxes represent δ sequences, which are the 335-bp long terminal repeats in Ty elements^{8,15,16}. **b**, Autoradiogram of *Xho*I restriction spectra from DNA of strain S288C and the five insertion mutants. The hybridization probe was nick-translated YIp333 which hybridizes in both parent strains to a 9.3-kb and a 20-kb *Xho*I fragment¹¹ (the latter did not transfer well). DNA of all mutant strains showed a new signal at around 3 kb. The corresponding new *Xho*I sites reside within the left δ sequences of the inserted Ty elements¹⁵. The *Xho*I fragment extending from the right δ sequences of the inserted Ty elements to the *Xho*I site upstream of the *LYS2* promoter can only be seen as weak signals in the 6-7-kb range due to the short homology with the probe. For orientation see Fig. 1a. The weak signals at around 5 and 10 kb are probably due to cross-hybridizing sequences elsewhere in the genome¹¹. **c**, Autoradiogram of *Xho*I-*Bgl*II restriction spectra in 1.2% agarose from DNA of the five insertion mutants. The probe was M13-650, which carries the *LYS2* promoter (see Fig. 1a). The numbers at the arrows designate fragment sizes in base pairs which were determined from co-migrating pBR322 *Hpa*II fragments³⁷. The size of 720 bp for the signal in *lys2*-655 DNA was independently determined from the pCH655 clone (see a) and was confirmed by DNA sequence analysis (D. Pridmore, personal communication).

Methods: The 5.6-kb *Bam*HI-*Sal*I fragment harbouring the *LYS2*-Ty1 junction of the mutant *lys2*-655 was cloned into the plasmid vector pBR322³⁸ after an enrichment step. 10 μ g of *Sal*I + *Bam*HI-cleaved *lys2*-655 DNA were electrophoresed on a 0.5% agarose gel and fragments of 5-7 kb electroeluted as described by Yang *et al.*³⁹. The yeast DNA was ligated with *Sal*I + *Bam*HI-cleaved pBR322 DNA which had also been purified by electroelution. Ampicillin-resistant transformants in *Escherichia coli* were screened for the presence of the 5.6-kb *Sal*I-*Bam*HI fragment by colony hybridization⁴⁰ using YIp333 as a probe. Sheared and denatured unlabelled pBR322 was added in excess to suppress hybridization between the labelled pBR322 sequences in the YIp333 probe and pBR322 DNA present in the *E. coli* transformants. One of the positive clones was picked and shown to contain the right plasmid, which was named pCH655.

Fig. 3 Map of Ty insertion sites. The insertion sites of the five Ty elements in the transcription start region of *LYS2* are indicated by arrows. The 4-kb long *LYS2* gene is drawn as black bar. The 5' end of this gene was determined from a DNA sequence analysis which identified the translation start (D. Pridmore and P.P., unpublished). The 3' end was determined by several independent S₁ mapping experiments¹¹. The 6-kb long Ty elements are not drawn to scale. Their insertion sites were calculated from the *Bgl*II-*Xho*I fragment sizes measured in Fig. 2c by taking into account the distance between the Ty end and the *Xho*I site in the δ sequence. This distance depends in the individual cases on the Ty orientation^{15,16} and is 240 bp for the two *lys2*aa mutants and 95 bp for the three *lys2* mutants. The arrows within the Ty elements correspond to the known Ty transcription direction³.



lead to a modulation of gene expression rather than to gene inactivation. This could be a molecular basis for the often postulated role of mobile DNA elements in evolutionary processes.

We thank Ursula Fleig for the m13-650 clone, Agathe Stotz for technical assistance, also Lucia Panzeri, Jürg Gafner and David Pridmore for many stimulating discussions, Tom Bickle for critically reading the manuscript and Elvira Amstutz for typing it. This work was supported by Swiss National Fund grant 3.644.80 and by a fellowship to H.E. from the Deutsche Forschungsgemeinschaft.

Received 17 August; accepted 22 November 1983.

1. Temin, H. M. *Cell* **21**, 599-600 (1980).
2. Eibel, H., Gafner, J., Stotz, A. & Philippsen, P. *Cold Spring Harb. Symp. quant. Biol.* **45**, 581-591 (1981).
3. Elder, R. T., Loh, E. T. & Davis, R. W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2432-2436 (1983).
4. Rubin, G. M. *et al. Cold Spring Harb. Symp. quant. Biol.* **45**, 619-628 (1981).
5. Flavell, A. J. & Ish-Horowitz, D. *Nature* **292**, 591-595 (1981).
6. Shiba, T. & Saigo, K. *Nature* **302**, 119-124 (1983).
7. Varmus, H. E. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 411-503 (Academic, New York, 1983).
8. Cameron, J. R., Loh, E. T. & Davis, R. W. *Cell* **16**, 739-751 (1979).
9. Chattoo, B. B. *et al. Genetics* **93**, 651-665 (1979).
10. Southern, E. J. *molec. Biol.* **98**, 503 (1975).
11. Eibel, H. & Philippsen, P. *Molec. gen. Genet.* **191**, 66-73 (1983).

12. Roeder, G. S. & Fink, G. R. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 299-328 (Academic, New York, 1983).
13. Williamson, V. M. *Int. Rev. Cytol.* (in the press).
14. Chattoo, B. B., Palmer, E., Bun-ichiro, O. & Sherman, F. *Genetics* **93**, 67-79 (1979).
15. Gafner, J. & Philippsen, P. *Nature* **286**, 414-418 (1980).
16. Farabaugh, P. J. & Fink, G. R. *Nature* **286**, 352-356 (1980).
17. Roeder, G. S. & Fink, G. R. *Cell* **21**, 239-249 (1980).
18. Roeder, G. S., Farabaugh, P. J., Chaleff, D. T. & Fink, G. R. *Science* **109**, 1375-1380 (1980).
19. Williamson, V. M., Young, E. T., Ciriacy, M. *Cell* **23**, 605-614 (1981).
20. Williamson, V. M., Cox, D., Young, E. T., Russel, D. W. & Smith, M. *Molec. cell. Biol.* **3**, 20-31 (1983).
21. Errede, B. *Cell* **25**, 427-436 (1980).
22. Montgomery, D. L. *et al. J. biol. Chem.* **257**, 7756-7751 (1982).
23. Jauniaux, J.-C., Dubois, E., Visser, S., Crabeel, M. & Wiame, J.-M. *EMBO J.* **1**, 1125-1131 (1982).
24. Scherer, S., Mann, C. & Davis, R. W. *Nature* **296**, 815-819 (1982).
25. Gafner, J., DeRobertis, E. & Philippsen, P. *EMBO J.* **2**, 583-591 (1983).
26. Goldberg, M. L. thesis, Stanford Univ. (1979).
27. Ikenaga, H. & Saigo, K. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4143-4147 (1982).
28. Snyder, M. P. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7430-7443 (1982).
29. Hayward, W. S., Neel, B. G. & Astrin, S. M. *Nature* **290**, 475-480 (1981).
30. Payne, G. S., Bishop, M. J. & Varmus, H. E. *Nature* **295**, 209-214 (1982).
31. Kuff, E. L. *et al. Nature* **302**, 547-548 (1983).
32. Schnicke, A., Harbers, K. & Jaenisch, R. *Nature* **304**, 315-320 (1983).
33. Messing, J. *3rd Cleveland Symp. Macromolecules: Recombinant DNA* (ed. Walton, A.) 143-153 (Elsevier, Amsterdam, 1981).
34. Smith, G. E. & Summers, M. D. *Analyt. Biochem.* **109**, 123-129 (1980).
35. Rigby, P. W., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237-251 (1977).
36. Philippsen, P., Kramer, R. A. & Davis, R. W. *J. molec. Biol.* **123**, 371-386 (1978).
37. Sutcliffe, J. G. *Cold Spring Harb. Symp. quant. Biol.* **43**, 77-90 (1978).
38. Bolivar, F. *et al. Gene* **2**, 95-113 (1977).
39. Yang, R. C. A., Liz, J. & Wu, R. *Meth. Enzym.* **68**, 178-181 (1979).
40. Grunstein, M. & Hogness, D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3961-3965 (1975).

Solution contact charging with respect to earthquake lights

LOCKNER *et al.*¹ have proposed a mechanism to explain the production of earthquake lights (EQLs) which strongly relies on the generation of electricity by the boiling of water in heated rocks in the shear zone. Using data from the original paper on saline charging² and spray electrification they explain the coronal origin of EQLs for magnitudes 6–7 but were unable to account for those of the Matsushiro swarm. More recent investigations of solution contact charging^{3,4} have demonstrated that the charging mechanism is not as Blanchard originally speculated, that is, “mechanical pulverization of water” with charge values similar to those of spray electrification, but is a solid-solid contact mechanism. Whereas sprayed aqueous solution droplets carry charges of about 4×10^{-19} C, those from solution contact boiling are in the range 3×10^{-17} to 3×10^{-14} C (refs 4, 5). The charges of 10^{-7} C g⁻¹ quoted from Blanchard related to saline solution undergoing transition boiling on a surface at approximately 350 °C. However, at temperatures cited by Lockner *et al.*, in excess of this, Leidenfrost boiling occurs to produce charges of 10^{-5} C g⁻¹. Although Leidenfrost boiling produces fewer droplets, each carries higher charge by factors of 3–5 above those on sprayed droplets.

I suggest that the higher values are more likely and may also account for the EQLs of the Matsushiro swarm, thereby bringing the latter within compass of the proposed mechanism. One further observation is that previous studies of electrification from boiling have been based on NaCl_{aq}. An extension of these investigations⁶ has revealed that many aqueous solutions including samples of groundwaters will generate electricity by flash, transition and Leidenfrost boiling.

The more recent information would seem to strengthen the mechanism proposed for the production of EQLs though some modification is required in which spray electrification should be deleted where boiling is involved and the more suitable solution contact charging process substituted.

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Carnivore social behaviour—does it need patches?

RECENTLY, Macdonald^{1,2} presented an interesting model explaining the evolution of social behaviour in carnivores. Briefly, the model, called the resource dispersion hypothesis (RDH), is as follows. Important food for carnivores occurs in patches and food availability in these patches varies with, for example, weather and season. Frequently situations occur when only one of these patches yields the resources needed for the animals' maintenance. Such situations can occur during single nights or seasons. Hence, the carnivore has to include enough patches within its territory (see, for example, Fig. 1 of ref. 1) to reliably support its minimum needs in all such situations. Territory size and configuration is determined by “dispersion of transient patches of available prey (ephemeral from night to night, or shifting from one season to the next)”¹. Group size, on the other hand, is positively correlated with the average patch quality (or quality of the least valuable patch, it is unclear which) (Fig. 1 of ref. 1).

Macdonald discusses predictions that are not necessarily borne out by the model, for example: “From the resource dispersion hypothesis one would not necessarily expect . . . any relationship between group size and territory area, as the two are argued to be affected, largely independently, by the abundance and dispersion of available food respectively”. The lack of such a correlation is then treated as a corroboration of the model¹. However, as RDH cannot be falsified regardless of whether there is a correlation or not, this prediction is useless for testing the hypothesis. Here I will identify and evaluate one prediction that is an *invariable* consequence of RDH: when the temporal variation is such that access to a given patch is vital to the territory owners, then all group members are expected to exploit this patch simultaneously. If the group members *regularly* forage in different patches simultaneously then the basic assumption of ephemerally unique food patches falls and so does RDH. This point was stressed by Bradbury and Vehrencamp³ when developing a model (very similar to RDH) to explain territory and group size in emballonurid bats. These authors³ found that all members of a given group of bats foraged in a common patch at any time.

Does it happen regularly that group members of, for example, badgers or red foxes forage simultaneously in a common patch? Macdonald's suggestion¹ that it may be “on a bad night or a bad year” makes it difficult to evaluate this prediction. Individual badgers move and hunt

solitarily inside a clan range⁴ and individual foxes are in physical contact with one another for as little as 4 min each night⁵. Also, Macdonald⁶ reported that dominant and subordinate red foxes in his study area outside Oxford generally foraged in different parts (and patches; Table 6 of ref. 6) of the group territory. Moreover, in the Revinge area in southern Sweden, breeding red foxes differed, in all seasons of the year, from their non-breeding group members in their use of hunting habitat⁷. Thus, on neither a nightly nor a seasonal basis do members of a given group seem to be restricted in their hunting to a given patch. It is not likely that a whole group should unite at a single food patch during a ‘bad’ year, unless one considers the entire territory as one patch, but then RDH is no longer valid. Macdonald¹ concludes that once group living in carnivores has evolved as an adaptation to resource dispersion then other benefits accruing from sociality *per se* (for example, cooperative hunting and defence of kills) further moulds the social organization in each population or species and that “the balance of these contemporary benefits is not necessarily the same as that which originally selected for group living”. Unfortunately, this *ad hoc* argument makes every serious attempt to test RDH virtually impossible as each time data that would falsify the hypothesis are obtained, it could always be claimed that a particular population is no longer under the original selection pressure for sociality. Even so, this would not help either for the Oxford or for the Revinge red foxes, as both populations are believed to be at an early stage in the evolution of sociality^{1,8}.

Recently^{8,9} I argued that permanently territorial altricial vertebrates that feed on an interannually fluctuating food resource, keep a constant territory size if the animals' lifespan is longer than the average time period of the food fluctuations. The animals' territory sizes will be so adjusted that they contain enough food for reproduction, or at least survival¹⁰, during the bottle-neck years of food scarcity. The model predicts that as food abundance increases, the territory owners, for example the breeding pair, take advantage of this resource surplus by allowing their adult offspring to remain at home, thereby increasing the group size. When food abundance decreases the subordinate group members will be evicted from the territory by the breeding pair. Hence, in any given territory of, say, foxes or badgers, group size will be positively correlated with the annual food abundance whereas territory size will be constant from year to year. This model does not include any assumption about ephemeral prey patches. A simple field test to distinguish

1. Lockner, D. A., Johnston, M. J. S. & Byerlee, J. D. *Nature* **302**, 28–37 (1983).
2. Blanchard, D. C. *Nature* **201**, 1164–1166 (1964).
3. Pounder, C. J. *Electrostat.* **1**, 395–397 (1975).
4. Pounder, C. J. *Electrostat.* **9**, 159–175; 177–182 (1980).
5. Bright, A. W. *Proc. R. Instn Gt Br.* **53**, 267–269 (1981).
6. Pounder, C. *Proc. Electrostat.* '83, *Inst. P. Ser.* **66**, 221–224 (1983).

between this model and RDH would be to prevent (for example, by a fence) groups of badgers or foxes exploiting a given food patch within their territories. My model predicts that territory size will remain constant if the experiment is performed in territories having three or more group members whereas group size will decrease in proportion to the ratio P_p/P_t , where P_p = prey productivity of the enclosed patch, and P_t = prey productivity of the entire territory. It is only when this experiment is performed in territories occupied by a single pair that the territory owners will try to expand or abandon their territories. Macdonald's^{1,2} model predicts that territory size will increase whereas group size may or may not remain constant (depending on the quality of the new food patch incorporated in the territory).

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1. Macdonald, D. W. *Nature* **301**, 379–384 (1983).
2. Macdonald, D. W. in *Proc. Worldwide Furbearer Conf. Vol. 2* (eds Chapman, J. & Pursley, D.) 918–949 (University of Maryland Press, 1981).
3. Bradbury, J. W. & Vehrencamp, S. L. *Behav. Ecol. Sociobiol.* **1**, 337–381; 383–404 (1976).
4. Kruuk, H. *J. Zool.* **184**, 1–19 (1978).
5. Macdonald, D. W. et al. in *A Handbook on Biotelemetry and Radio Tracking* (eds Amlaner, C. J. & Macdonald, D. W.) 405–424 (Pergamon, Oxford, 1980).
6. Macdonald, D. W. *Z. Tierpsychol.* **52**, 171–200 (1980).
7. von Schantz, T. *Oikos* **37**, 63–68 (1981).
8. von Schantz, T. *Oikos* **42**, 48–58; 59–65 (1984).
9. von Schantz, T. thesis, Univ. Lund (1981).
10. Lindström, E. thesis, Univ. Stockholm (1982).

MACDONALD REPLIES—Although most easily visualized where only one patch is likely to be fruitful in each territory at a given time (refs 1 and 2; hence Fig. 1 in ref. 3), the resource dispersion hypothesis (RDH) does not require these conditions^{4–8}. Rather, the hypothesis identifies conditions of resource dispersion where “the smallest home range with an economically defensible” configuration which will reliably support a pair of foxes (on a bad night or a bad year) may sometimes support additional foxes¹³.

In spatially patchy environments, convoluted territorial borders might encompass sufficient fragments of transient patches to yield food for only a pair of residents. RDH proposes that the defence of such ranges may not be economic⁹ whereas a less convoluted configuration, perhaps including more resources, could be defensible. On this view, each territory may contain many patches, each being variously fruitful at any one time. Whether group members forage in a patch simultaneously, just as whether they travel and/or forage alone or together, will depend on the nature of the prey and on other selective pressures affecting their sociality³.

I have suggested⁸ that where prey were spatiotemporally uniform in their availability, there the smallest economically

defendable range that could sustain a pair would not support additional adults (whether or not, and for how long, it would be advantageous to maintain territories larger than this minimum size would depend on such factors as the magnitude of pressure from intruders and the benefits of increasing group size). von Schantz's formulation concerns an intermediate case where, irrespective of its dispersion, prey is temporally variable. Again, the pattern of prey availability creates conditions where the minimum range which supports a pair (in a poor year) can sometimes support extra adults (in a rich year). The interannual mode^{10,11} highlights the question of how social behaviour provides “a buffer to prevent spatial organization altering prematurely in response to ephemeral changes in food availability”⁸. von Schantz's¹⁰ emphasis on the costs of territorial readjustment is compatible with my formulation.

Territory size and shape may sometimes be adapted to ‘bottle-neck’ periods in the abundance of available prey; it seems no less plausible (nor at variance with the data^{4–8} to argue that they are sometimes further constrained by the dispersion of resources. von Schantz is mistaken in thinking that I treated independence of territory and group sizes as corroborating RDH; rather, I showed how this was accommodated by RDH. Experiments like that suggested are vital although the result would depend on which patch was fenced (due to interacting variation among dominance and patch richness and utilization¹²).

von Schantz's hypothesis requires group size to descend to two during the average breeding lifespan. Among species which form “spatial groups”³, these conditions are probably not met in some carnivore populations¹³, although they may be in others^{6,10}. The spatiotemporal formulation of RDH is applicable to groups of stable or unstable membership whereas the purely temporal formulation may explain only those unstable groups whose membership periodically decreases to two. Some badger and fox groups reported in refs 4, 5, 8 and 13 have subsequently remained larger than 2 for over 10 yrs (H. Hofer, D. W. M., E. Neal and J. Phillipson, unpublished observations).

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1. Bradbury, J. W. & Vehrencamp, S. L. *Behav. Ecol. Sociobiol.* **1**, 383–404 (1976).
2. Clutton-Brock, T. H. *Nature* **250**, 539–542 (1974).
3. Macdonald, D. W. *Nature* **301**, 379–384 (1983).
4. Kruuk, H. *Behav. Ecol. Sociobiol.* **4**, 75–89 (1978).
5. Kruuk, H. & Parish, T. J. *J. Zool.* **196**, 31–39 (1982).
6. Mills, M. G. L. *J. Zool.* **198**, 39–51 (1982).
7. Hersteinsson, P. & Macdonald, D. W. *Symp. zool. Soc. Lond.* **49**, 259–289 (1982).
8. Macdonald, D. W. in *Proc. Worldwide Furbearer Conf. Vol. 2* (eds Chapman, J. & Pursley, D.) 918–949 (University of Maryland Press, 1981).

9. Brown, J. L. *Wilson Bull.* **70**, 160–169 (1964); *J. theor. Biol.* **95**, 793–810 (1982).
10. von Schantz, T. thesis, Univ. Lund (1981); *Oikos* **42**, 48–58; 59–65 (1984).
11. Lindström, E. in *The Red Fox* (ed. Zimen, E.) 177–184 (Junk, The Hague, 1980).
12. Macdonald, D. W. *Z. Tierpsychol.* **52**, 171–200 (1980).
13. Neal, E. *Badgers*, 321 (Blandford, Poole, Dorset, 1977).

111-day periodicity of X-ray transient A0535+26

PRIEDHORSKY AND TERRELL have recently argued about some special characteristics of X-ray transient A0535+26 (ref. 1) to justify their speculations: that (1) the optical counterpart HDE245770 of A0535+26 cannot be a BO Ve star; (2) it would be better if the terminal velocity of the stellar wind from HDE245770 were $<1,000 \text{ km s}^{-1}$; (3) the B star must be rotating rapidly to lose matter from the equatorial region; (4) the companion HDE245770 must have a mass loss rate of $\sim 10^{-5} M_{\odot} \text{ yr}^{-1}$ to power the X-ray luminosity of A0535+26.

Furthermore, they point out that there is a discrepancy between evaluations of the mass loss rate from UV data and their own estimates (see point (4)).

These five points have been discussed elsewhere. Specifically: (1) HDE 245770, optical counterpart of A0535+26 (ref. 2), is an O.9 IIIe star³; (2) the terminal velocity of the stellar wind in HDE245770 is $\sim 630 \text{ km s}^{-1}$ (ref. 4); (3) from the broadening of He(II) line (1,640 Å), the rotational velocity of HDE245770 is $v_e \sin i \approx 230 \text{ km s}^{-1}$ (ref. 5); (4) the mass loss rate of HDE245770, derived by IR data, is of the order of $10^{-6} M_{\odot} \text{ yr}^{-1}$ (ref. 6); (5) the discrepancy between evaluations of the mass loss rate derived from UV and IR data has been discussed in refs 5, 7.

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1. Priedhorsky, W. C. & Terrell, J. *Nature* **303**, 681 (1983).
2. Bartolini, C., Guarnieri, A., Piccioni, A., Giangrande, A. & Giovannelli, F. *IAU Circ. No.* 3167 (1978).
3. Giangrande, A., Giovannelli, F., Bartolini, C., Guarnieri, A. & Piccioni, A. *Astr. Astrophys. Suppl.* **40**, 289 (1980).
4. Giovannelli, F. et al. *Space Sci. Rev.* **30**, 407 (1981).
5. Giovannelli, F. et al. *Proc. 3rd European IUE Conf. ESA SP-176*, 233 (1982).
6. Persi, P. et al. *Mon. Not. R. astr. Soc.* **187**, 293 (1979).
7. Snow, T. P. *Be Stars, Proc. IAU Symp. No. 98*, 509 (1982).

PRIEDHORSKY AND TERRELL REPLY

—We thank Giovannelli for pointing out references relevant to our letter, some of which we may have overlooked even if space had permitted their inclusion. We had intended only to present new X-ray observations of A0535+26. It is satisfying that the IR studies cited by Giovannelli support our conclusion that the mean mass loss rate of HDE245770 to its companion A0535+26 is of the order $M \sim 10^{-5} (v_{\text{wind}}/1,000 \text{ km s}^{-1})^4 M_{\odot} \text{ yr}^{-1}$.

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Consciousness and conscience

Stuart Sutherland

Consciousness Regained. By Nicholas Humphrey.
Oxford University Press: 1983. Pp.222. £12.95, \$19.95.

MANY of the world's best minds have wrestled with the problem of consciousness; so for that matter have many of the worst. In *Consciousness Regained*, a collection of essays most of which have been published elsewhere, Dr Nicholas Humphrey joins the throng. He claims, unexceptionally, that since consciousness has presumably evolved, it must have a function, which is, he suggests, to enhance social interaction. He reaches this conclusion in two steps, the first of which concerns the intellect rather than consciousness.

According to Humphrey, the intellect in man and many other primates has been developed to a point far beyond that needed to deal with the material environment. He argues that chimpanzees and gorillas, who have invented a technology for subsisting, have less need for intelligence than do less technologically orientated primates. Merely by watching another member of its group, a chimpanzee can learn to poke a stick into a termites' nest and draw it out covered with a tasty feast. The individual animal does not have to use creative intelligence. The argument ignores the question of how the technique was invented in the first place: even if it was learned by trial and error, poking sticks into nests is a creative act and the chimpanzee has to make the connection between it and the resulting mouthful of termites. Moreover, although high intelligence might not be needed for day-to-day living, chimpanzees and gorillas must sometimes be subjected to novel and dangerous events which they can survive only by the use of intelligence. In the case of man, the argument is even weaker since his physical defences and resources are less and his survival — often under very adverse external conditions — depends entirely on his ability to manipulate the physical world by the use of intelligence. Although it is impossible to assess how far the intelligence of a given species evolved through pressure to cope with the physical world, there may be some truth in Humphrey's ingenious alternative — that in man and some other primates it evolved in order to help the individual deal with other members of the species.

There is, however, a big leap from this conclusion to the next step in his argument: he maintains that consciousness itself arose in order to help man (and possibly some other primates) understand the behaviour of others. Consciousness provides a model of the way in which people's own minds work — a model of their emotions,

motives, thoughts and sensations: in so far as different minds are similar this model provides a basis for understanding others. There is, unfortunately, an obvious fallacy in the argument. The brain could represent the processes underlying motives, thinking and so on and could use this representation as a model for others' behaviour and the forces underlying it without the representation appearing in consciousness. Indeed,

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Nicholas Humphrey — "fun to read".

Humphrey himself argues elsewhere that the brain contains a representation of the position of the parts of the body, but that this representation does not appear in consciousness. It is easy to invent functions that consciousness might subserve: what is difficult and has never yet been achieved is to show that they can be subserved only by consciousness.

There are three lesser problems with Humphrey's thesis. First, he does not sufficiently distinguish between consciousness and self-awareness. It is hard to believe that species other than primates lack consciousness, though they may lack self-awareness. Second, it is simply not true that in most of their dealings with others people use a conscious model either of their own behaviour or of that of others. People normally interact spontaneously and with

little or no conscious thought. It is only when social interactions go wrong that they start to think consciously about them — often with unfortunate consequences. Third, if the reason why people are self-aware is to enable them to understand others, it is curious that self-knowledge should be so fallible. People's motives are often more transparent to others than to themselves.

For the rest, Humphrey is always stimulating and fun to read, though by its nature most of his book is rather bitty. He develops the far from novel idea that the reason why people engage in activities which seem to have no direct relevance to survival is that the activities provide novel experiences in terms of which people can better understand others. Some examples are play, initiation ceremonies, dreaming, keeping pets, watching dramatic performances and the manipulation of children by parents — particularly gratuitous cruelty. It is hard to accept that the function of such cruelty is to teach a child what it feels like to be unfairly treated, particularly as what evidence there is suggests that maltreated children tend to become poorly adjusted adults.

Another perennial problem that Humphrey tackles is that of the value of art. He argues that in classifying objects in the natural world, people look for sets whose members are similar in some respect to all the others, but differ in some way from the members of other sets. Much art (and, he might have added, almost all music) consists of variations on a theme and hence it teaches the viewer to spot similarities and differences and gives him practice in the task of classification. This is an ingenious account of the intellectual content of art, but leaves out its emotional force.

The book ends with a series of disconnected essays and book reviews, which are lively, but lightweight. Humphrey rightly and suavely demolishes the pretensions of such self-appointed gurus as Douglas Hofstadter and Gregory Bateson, and he provides a piece of impassioned rhetoric on nuclear war, in which he appeals to everyone to go out and stop it. He declaims "I have not heard of one good reason for not halting [nuclear armaments] tomorrow". Unfortunately he is so carried away by his own rhetoric that he omits to tell both the man in the street and the politicians exactly what it is they should do to prevent nuclear war.

Humphrey writes with elegance and force, and although he sometimes fails to follow his ideas through, they are always stimulating. Even the reader who disagrees with his arguments will derive pleasure from working out for himself why they are wrong. □

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Tackling the supreme symmetry

M. J. Duff

Superspace or One Thousand and One Lessons in Supersymmetry.

By S.J. Gates Jr, M.T. Grisaru, M. Roček and W. Siegel.

Benjamin/Cummings: 1983. Pp.548. Hbk \$48, £40.80; pbk \$25, £21.25.

HAD Scheherazade tried to while away those Arabian nights with *One Thousand and One Lessons in Supersymmetry*, one has the feeling that long before the chapter on quantum superspace Schahriar would have cried "Hey, wait a minute! Where are the references?". The graduate student of theoretical physics or other inquisitive researcher, hoping to use this text as a guide to the literature on supersymmetry and supergravity, will be sadly disappointed. Not one reference to the original works is to be found in the book. And of the six references to conference proceedings, one is incorrect. (The title of the 1981 Trieste proceedings should be *Supergravity 81* and the editors were S. Ferrara and J. G. Taylor.) The authors, all of whom are well-known Sultans of Supersymmetry, apologize to their friends in the field and beg pardon for omitting the credits. While I suspect that no such pardon will be forthcoming, this is nevertheless a valuable addition to the introductory literature on the ideas of supersymmetry and superspace which will be essential reading for the devotee.

In the words of the Introduction, "Supersymmetry is the supreme symmetry: It unifies spacetime symmetries with internal symmetries, fermions with bosons, and (local supersymmetry) gravity with matter". About one-third of the current publications in theoretical elementary particle physics is devoted to supersymmetry or supergravity, and an increasing number of experimental papers deal with the search for the still unobserved (and perhaps unobservable) superparticles which the theory predicts. More and more PhD students are choosing this field for their research yet constantly one hears the complaint that there is no adequate starting point for the learner. *Superspace* will certainly be of help, but it is not for the casual browser or the dilettante.

Supersymmetry experts fall into two categories: those who treat the theory like any other quantum field theory, using separate "component" fields to describe both bosonic (integer spin) and fermionic (odd-half-integer spin) particles; and those who prefer the new-fangled approach in which bosons and fermions are combined into "superfields" which live in a strange world called "superspace". In superspace, the four spacetime dimensions are bosonic

with coordinates obeying the usual multiplication $AB = +BA$ but extra fermionic dimensions are also introduced whose coordinates obey $AB = -BA$.

As their title suggests, the authors of *Superspace* are unashamedly in favour of these superfields and use the superspace formalism throughout. They have covered all aspects of superspace: superspace geometry, representations of supersymmetry, and global superfields, but their ultimate goal is the unification of gravity and matter via supergravity. At present supergravity is the best available candidate for the Unified Field Theory which will unite gravity with the other fundamental forces of nature. One of the problems in

such a scheme is to avoid the ultraviolet divergences of quantum gravity. The authors present a convincing case that only with the techniques of super-Feynman diagrams (for which they themselves have been largely responsible) can we hope to answer this all-important question.

Superspace is an Aladdin's cave of highly-condensed information. But although it is written with an easy style and admirable clarity, it will require a keen mind and determination, rather than a simple "Open, Sesame", to extract the riches. □

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Jenner by himself

Donald R. Hopkins

Letters of Edward Jenner: And Other Documents Concerning the Early History of Vaccination.

Edited by Genevieve Miller.

Johns Hopkins University Press: 1983. Pp.145. \$15, £12.75.

THE recent global triumph over smallpox has stimulated great interest in historical aspects of that disease and persons associated with it. So it is the right time finally to have published these letters of Edward Jenner from the Henry Barton Jacobs Collection at Johns Hopkins University. More than anyone else, it was Jenner who set the stage for the eventual eradication of smallpox with his epochal discovery of vaccination in 1796.

Because of Jenner's great historical im-

portance and his broad interests, I have often wished I could hear him describe his discovery and the related events that followed, at first-hand, or at least in a taped interview. Fortunately, even though Jenner is of an earlier era and we have no taped interview, we do have many of his letters, some 600 of which are known to still exist. This book contains a good many (110) of them, most being published here for the first time.

As Genevieve Miller notes in her brief introductory essay, in these letters to 45 different correspondents we meet Jenner the naturalist, author, father, country gentleman, scientist, husband, horticulturalist, geologist and the medical practitioner. We also meet a man who, like all of us, was occasionally wildly mistaken. Most interestingly, however, since all but two of these letters are from the period after he discovered vaccination (when he was 47 years old and already had made several significant scientific discoveries

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Cow pock and the fears of the public — Gillray's celebrated cartoon of 1802, lampooning the activities of the Anti-Vaccine Society.

which were overshadowed by his later work), we meet the humanist Father of Vaccination. From 1796, until his death in 1823, Jenner constantly and doggedly promoted his discovery in the face of persistent opposition.

Some of these encounters seem to me to have almost as much force as if they were face to face. We feel Jenner's exaltation, for example, when he learns of the successes of vaccination in Copenhagen and Ceylon, and of the spectacular expedition dispatched by the King of Spain under Francisco Xavier Balmis which brought vaccination to Spanish territories around the world:

A few days since, I rec'd from Madrid a Document respecting Vaccination which fills me with more astonishment than anything that has yet reach'd me on that subject . . . Would to Heaven the British Cabinet had shown the same Philanthropic Spirit as that of Spain! [p.31].

We also share his exasperation and frustration at the cost in lives and suffering as a result of the antivaccinists in his own country, while much of the rest of the world adopted his discovery with thankful alacrity:

One would think the statement of Facts, as they now stand before the Public from every quarter of the Globe would blow away such stuff as these abominable people produce, like Chaff, but it is not so, or the Bills of Mortality would not exhibit weekly such horrid scenes of devastation from the Smallpox [p.70].

No biographer or historian could convey Jenner's feelings during this period of his life better than he does himself in these letters.

The format of the book is well chosen. The annotated letters are presented in chronological order, except for the six which are of unknown date. Miller introduces the correspondents, and other persons as they are first mentioned, with short biographical sketches. In helpful but unobtrusive footnotes, she also explains various phrases, objects, practices and so on which are unfamiliar to us. Easy access to the contents is assured by means of an index, and by the table of contents in which Miller lists the letters in order, with the name of the correspondent and the key subject(s) discussed in each.

Fifteen other documents which are included in the 20-page appendix, we are told, "constitute the remainder of the unpublished Jacobs material relating to Jenner or to contemporaries involved with vaccination" (p.xxvi).

My only real disappointment after reading the book is a direct result of its effectiveness: I came away yearning to see all of Jenner's letters compiled and published in a single work. Until then, however, this collection will do very well. □

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All of physics

R.H. Dalitz

McGraw-Hill Encyclopedia of Physics.

Editor-in-chief Sybil P. Parker.

McGraw-Hill: 1983.

Pp. 1,337. \$59.50, £43.75.

The McGraw-Hill Encyclopedia of Science and Technology is a well-known publication, unique in its coverage of all fields of science. It has been revised about every five years, the most recent edition being the fifth which was published in 1982 (for review see *Nature* 229, 283; 1982). Its articles have developed over the years, so that they are now well pruned of excess material and unusually free from errors.

This McGraw-Hill Encyclopedia of Physics has been made up by selecting 760 articles dealing with aspects of physics and mathematics, taken from the 1982 edition of the larger work. About 40 per cent of these articles are quite short, occupying less than a column but with ample cross-reference to the longer pieces in which they find a natural place. About 12 per cent are more substantial, occupying more than 8 columns each. Some of these are much longer, for example *gas dynamics* (15 columns), *superconductivity* (17), *radioactivity* (35) and *ultrasonics* (15). To categorize the articles in this way is perhaps misleading, however, since some large topics have been broken up; thus for fluid flow we have *fluids* (4), *fluid dynamics* (1), *fluid flow* (9), *fluid flow principles* (2), *fluid mechanics* (3) and *fluid statics* (1), not to mention *hydrodynamics* (8), *turbulent flow* (5), *magnetohydrodynamics* (11) and *gas dynamics* (15). On the other hand, *particle accelerator*, amounting to 50 columns, is a single entry but has seven sections, each with a different author. Perhaps more of the shorter pieces could have been grouped together in this way, although this might have made them appear forbidding to inexperienced readers.

Many of the articles are excellent. Plasma physics and nuclear physics are particularly well-served, the accounts being quite up to date and clearly illustrated, but the areas of elementary particle physics and condensed matter are also very well covered, as are more traditional topics in articles such as *units of measurement*, *sound*, *molecular physics* and *gravitation*. The mathematical articles are compact but generally quite accessible. Their style varies considerably; for example *combinational theory*, *graph theory*, *statistics* and *trigonometry* are clear and readable whereas a few of them, while being excellent and authoritative, are rather technical for the average physicist, let alone a high-school student. All but the shortest pieces have an adequate bibliography, two to five sources being quoted, generally covering standard books on the subject as well as one or two recent

review articles. The articles are well cross-referenced, often paragraph by paragraph, and at the end of the volume there is a detailed index.

The reader will generally start with the index, which will offer a possible article. In reading this, he will find cross-references to related articles. By proceeding systematically in this way, he will generally be led to what he needs. For example, I tried the term *eigenvalue*, for which there is no article. The index referred me to *quantum mechanics* and to *eigenfunction*, while the latter referred me to the former and to *non-relativistic quantum theory*, so I quickly reached my goal. However, it is possible for topics to slip through this overlapping net. For example, spontaneous decay of the proton, a central topic of current research, is not in the index. The article *proton* does not mention it. The index for proton lists *proton radioactivity* but this turns out to be nuclear decay by the spontaneous emission of a proton. After some looking around, I found the topic contained in the *baryon* article under the subheading *proton stability*, and in the *elementary particle* article under the subheading *grand unified theories*. Another example is cosmic radiation, although this subject may perhaps be considered more a part of geophysics or astrophysics. The index led me to *bremstrahlung*, in which proton emission by cosmic ray particles is briefly mentioned. This article gives a cross-reference to *cosmic rays*, but there is no article of that title. The basic facts about cosmic rays really merit a place in this volume, since they have been historically of immense importance and still give the highest-energy nuclear collisions available for study.

It is natural to ask how this encyclopaedia compares with the *Encyclopedia of Physics* published by Addison-Wesley in 1981 (for review see *Nature* 290, 657; 1981). The main difference is that the latter is really intended for other physicists, or at least for scientists in neighbouring fields, who seek enlightenment on a topic peripheral to their own area of expertise. It has fewer articles but they are longer, on average, and considerably more technical, with equations being used freely. However, it seems that many of the articles of the Addison-Wesley encyclopaedia were written not later than early 1977, so it is not up to date on topics in areas of rapid growth. The McGraw-Hill version would appear to be the most suitable available for the audience to which it is directed — high schools and colleges. But all concerned with mathematics, physics, chemistry or engineering will find much of interest to them in its pages, laid out in an attractive and easily digestible form. □

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Astrophysical fruit

Geoffrey Bath

The Left Hand of Creation: The Origin and Evolution of the Expanding Universe.

By John D. Barrow and Joseph Silk.
Basic Books/Heinemann: 1983. Pp. 256.
\$17.95, £9.95.

"THEORETICAL astrophysics is as exact a science as geometry", remarked the Oxford mathematician E. A. Milne in a paper in 1930. Milne's affirmation in the exactitude of astrophysics followed introductory remarks outlining the problem of radiative transfer through a spherical shell of gas. This introduction concluded with an outspoken defence of what Milne saw as one of the chief necessities for progress — namely, the need to simplify a complex problem into an analytically tractable set of conditions, in his case the simultaneous and self-consistent solution of radiative transfer through matter, including ionization and excitation. Milne confined his attention to "the simplest question that can be propounded". That is, the case of static grey material. Today those working in the field of radiative transfer are approaching the solution of non-LTE (local thermodynamic equilibrium), extended, relativistically expanding atmospheres. But no one working in the field would deny the truth of Milne's justification for a simpler approach in 1930. "Until we have fully and rigorously discussed the case of grey material we are unlikely to make useful progress with more complicated hypotheses."

It is salutary to reflect on the state of model atmosphere theory half-a-century ago in the context of developments within modern cosmology. In the past cosmology has all too frequently been dismissed as an insular, acroamatic subject; a science which, with only the one singular case open to investigation, was doomed to failure on the grounds of inadequate experimental verification. For after all, so the argument goes, how could Newton possibly have succeeded in his theory of gravitation with only the one instance of a single apple falling on his head as experimental proof? Amusing though the myth may be, it was the extensive studies of motion and dynamics for two thousand years, from Aristotle and Richard of Wallingford to Galileo and Newton himself, which permitted a precise theory of gravity to be formulated.

However to view cosmology as the study of one experiment of the Grand Designer is to mistake the vessel for the cargo it contains. Cosmology is not the science of one single aspect of the Universe. Rather it is all aspects, woven against the tapestry of time, space and the forces of nature. The attempt to understand the origin, development and ultimate fate of its constituents is

no different in principle from the aim of chemistry to unravel the code of chemical bonding, or of molecular biology to tease out the molecular basis of life.

Barrow and Silk present this quest with wit and gusto. Confident and experienced guides, they conduct their readers around the great debates in contemporary cosmology. At issue are such questions as the origin of the lack of antimatter, the source of spatial fluctuations responsible for all present structure in the Universe, the quasi-supernatural, as they seem superficially, properties of the vacuum giving rise to the paradox of black hole evaporation, and the progressive decomposition of the cosmic soup as time is run backwards towards the Planck time when the Universe was 10^{-43} seconds old, complete symmetry held sway, and all forces were indistinguishable.

No new development in fundamental physics can take place today without its influence on the evolution of the Universe being tested. Increasingly cosmology, and the astronomical evidence at its core, plays

a fruitful role as a guide to permissible physical theorizing, supplementing more orthodox laboratory experiment and verification. The resulting astrophysical theorems aim at an ultimate amalgamation of theory and observation which will account for the form and content of the Universe — a Universe seen as a necessary consequence of physical law, with only the most basic axioms as the starting point.

But do not turn to this book for proofs of those theorems. The excitement and challenge are captured, but the rigour is necessarily elsewhere. Just as for many young minds the bald statement of Pythagoras's theorem is the first step towards a clearer perception of the structure underlying the physical world, so this book is a primer to recent attempts at unification of the forces of nature, and the powerful patterns those attempts are creating in the science of astrophysics. □

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Fresh yeast

D.R. Harper

Yeasts: Characteristics and Identification.

By J.A. Barnett, R.W. Payne and D. Yarrow.

Cambridge University Press: 1983.
Pp. 811. £75, \$150.

MANY of the outstanding publications dealing with the classification, nomenclature and identification of yeasts have, in the past, come from the Centraalbureau voor Schimmelcultures in Delft. Jacomina Lodder, of the Centraalbureau, edited what has for many years been considered as the definitive work on yeast taxonomy, *The Yeasts: A Taxonomic Study* (North-Holland, 1970), in which she provided the characteristics of 349 yeast species and described relevant laboratory methods. However, her scheme for identification placed emphasis on the ability of organisms to produce ascospores. This led, inevitably, to practical problems associated with demonstrating sporulation.

In England, J.A. Barnett and R.J. Pankhurst approached yeast identification in a different way, and in *A New Key to the Yeasts* (North-Holland, 1974) presented a computer-derived key which involved only physiological tests. *A New Key* was based on the information given in *The Yeasts* and was intended to be used in conjunction with it.

Five years later, *A Guide to Identifying and Classifying Yeasts* (Cambridge University Press, 1979) brought together as authors J.A. Barnett and R.W. Payne, with D. Yarrow from the Centraalbureau. Improved methods of computing were used to generate identification keys consisting of either physiological tests or both

physiological and morphological observations. However, it was again necessary to consult Lodder's text for certain additional details.

The successor to *A New Key* and *A Guide* is *Yeasts: Characteristics and Identification*, an up-to-date book which does not rely on another work for any essential information. In it, Barnett, Payne and Yarrow discuss yeast classification and give commonly used procedures for yeast identification. In addition, they provide detailed accounts of 469 yeast species; responses to 83 tests and clear photomicrographs are also included.

The authors have revised the successful format of *A Guide* and incorporated it into this volume. They give 18 identification keys which deal with various groups of yeasts (for example, clinical isolates, yeasts associated with foods and beverages, and yeasts with particular physiological or morphological attributes). Lists of small numbers of tests for the identification of single species are provided. One chapter presents generic characteristics and another conveniently tabulates the characteristics of each species. The extra sections containing specific epithets and yeast names will undoubtedly prove invaluable given the confusion caused by nomenclatural changes in yeast taxonomy.

In producing this book, Barnett, Payne and Yarrow have met the daunting standards set by Lodder in 1970. *Yeasts: Characteristics and Identification* satisfies the requirements of being comprehensive and of containing easily accessible information. It will undoubtedly stand as the major text on yeast taxonomy for a considerable time to come. □

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Psychology

BURROWS, G.D., NORMAN, T.R. and DAVIES, B. (eds). *Antidepressants. Drugs in Psychiatry*, Vol.1. Pp.350. ISBN 0-444-80474-9. (Elsevier Biomedical/North Holland: 1983.) \$45, Dfl.130.

CLARK, J.H. *A Map of Mental States*. Pp.242. Pbk ISBN 0-7100-9235-0. (Routledge & Kegan Paul: 1983.) £6.95.

IVERSEN, L.L., IVERSEN, S.D. and SNYDER, S.H. (eds). *Neuropeptides*, Vol.16. *Handbook of Psychopharmacology*. Pp.577. ISBN 0-306-41048-6. (Plenum: 1983.) \$65.

KILMANN, P.R. and MILLS, K.H. *All About Sex Therapy*. Pp.227. ISBN 0-306-41317-5. (Plenum: 1983.) \$15.95.

OLLENDICK, T.H. and HERSEN, M. (eds). *Handbook of Child Psychopathology*. Pp.526. ISBN 0-306-40938-0. (Plenum: 1983.) \$50.

REASON, J. and MYCIELSKA, K. *Absent-Minded?: The Psychology of Mental Lapses and Everyday Errors*. Pp.262. Hbk ISBN 0-13-001743-4; pbk ISBN 0-13-001735-3. (Prentice-Hall: 1982.) Hbk np; pbk £5.90.

RIEBER, R.W. (ed.). *Dialogues on the Psychology of Language and Thought*. Pp.166. ISBN 0-306-41185-7. (Plenum: 1983.) \$19.50.

SMITH, T. *The Homeopathic Treatment of Emotional Illness: A Self-help Guide to Remedies Which Can Restore Calm and Happiness*. Pp.208. Pbk ISBN 0-7225-0812-3. (Thorsons: 1983.) £5.95.

TALBOTT, J.A. and KAPLAN, S.R. (eds). *Psychiatric Administration A Comprehensive Text for the Clinician-Executive*. Pp.410. ISBN 0-8089-1529-0. (Grune & Stratton: 1983.) \$38.50.

Biological Sciences

MURAMATSU, T. *et al.* (eds). *Teratocarcinoma and Embryonic Cell Interactions*. Pp.344. ISBN 4-7622-5294-8/0-12-511180-0. (Japan Scientific Societies Press/Academic: 1982.) \$36.

NADELSON, C.C. and MARCOTTE, D.B. (eds). *Treatment Interventions in Human Sexuality*. Pp.488. ISBN 0-306-41082-6. (Plenum: 1983.) \$42.50.

NOAKES, D.L.G. *et al.* (eds). *Predators and Prey in Fishes. Proceedings of the 3rd Biennial Conference held May 1981, Illinois*. Pp.230. ISBN 90-6193-922-8. (Dr W. Junk: 1983.) Dfl.150, \$65.

NOGGLE, G.R. and FRITZ, G.J. *Introductory Plant Physiology*, 2nd Edn. Pp.627. ISBN 0-13-502096-4. (Prentice-Hall: 1983.) £27.85.

OPLATKA, A. and BALABAN, M. (eds). *Biological Structures and Coupled Flows*. Pp.519. ISBN 0-12-527520-4/0-86689-016-5. (Academic/Balaban ISS: 1983.) Np.

PARRISH, J.A. (ed.). *Photoimmunology*. Pp.304. ISBN 0-306-41280-2. (Plenum: 1983.) \$39.50.

PAUL, W.E. *et al.* *Annual Review of Immunology*, Vol.1. Pp.666. ISBN 0-8243-3001-3. (Annual Reviews Inc: 1983.) \$27 (US), \$30 (elsewhere).

PAVONE-MACALUSO, M. and SMITH, P.H. (eds). *Cancer of the Prostate and Kidney. NATO Advanced Science Institute Series*, Vol.53. Pp.750. ISBN 0-306-41132-6. (Plenum: 1983.) \$89.50.

PENTZ, L. *The Biolab Book: Twenty-six Laboratory Exercises for Biology Students*. Pp.127. Pbk ISBN 0-8018-2512-1. (Johns Hopkins University Press: 1983.) £8.50, \$12.95.

PIANKA, E.R. *Evolutionary Ecology*, 3rd Edn. Pp.416. ISBN 0-06-045232-3. (Harper & Row: 1982.) £20.

Pocket Life Sciences Dictionary. Pp.270. Pbk ISBN 0-582-55656-2. (Longman: 1983.) 90p.

PRUDHOE, S. *British Polyclad Turbellarians: Keys and Notes for the Identification of the Species*. No.26. Pp.77. Hbk ISBN 0-521-25545-7; pbk ISBN 0-521-27076-6. (Cambridge University Press: 1982.) Hbk £12.50; pbk £5.95.

PURKHIT, S.S. (ed.). *Aspects of Physiology and Biochemistry of Plant Hormones*. Pp.345. No ISBN. (Kalyani, New Delhi: 1983.) Rs.35.

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Image analysis

This week's feature focuses on recent developments in image analysis systems

● Spectragraphics has released a new type of computer graphics workstation: the system 1500 Multi-Station. The system 1500 provides a range of interactive graphics features as well as a raster graphics workstation. The system's controller supports from one to four workstations. When configured with a 'communications controller', the system 1500 can support up to 64 workstations. It is offered with a choice of 32-bit parallel interfaces for the IBM, Perkin-Elmer, and Digital Equipment host processors. The system 1500 Multi-Station is designed to accommodate graphics applications such as: computer aided design/computer aided manufacture (CAD/CAM), signal processing, seismic interpretation, command and control, simulation, mapping and molecular modelling. It is available in either monochrome or colour (16, 256, or 4096 simultaneous colours from palettes with up to 16 million colours). The workstation's cathode ray tube is a raster-scan 1024×1024 display. Objects can be rotated, scaled and translated. Multiple viewports can be defined and transformed images clipped to their boundaries at real-time rates. In addition, a range of input peripherals is available. This includes keyboards, joysticks, data tablets, digitizers, programmable function switches and programmable function dials.

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● A series of image analysis systems is available from Artek. The system 810 is modular and is designed such that all the components are able to interface with peripherals and computers. The second system, the model 982, is Artek's basic image analysis building block. It is designed for use in particle sizing, producing histograms, edge or density counting, area measurement and microscopic applications. Also available is the digitized video system with the 940 memory and image editor. This consists of the video from the model 982 which is digitized into a 512 × 512 × 1 format. The computer functions it can perform include selecting and rejecting objects, filling holes and drawing a measuring frame to match a sample.

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● Two new 6-bit and 8-bit video frame memories have been manufactured by Hamamatsu and are available in the UK through Hakuto International. The units, designated the models C1900 and C1900-01, will digitize and store analog video signals from a television camera and will provide video outputs of the stored image in real time. The C1900 has a 6-bit memory (64 levels) for storing the intensity level of each picture element. It is intended for use with general television cameras as well as Hamamatsu's own range, the C1000 series. The C1900-01 has an 8-bit memory (256 levels) and its own synchronous generator. The camera head of the C1000 or C1145 series non-interlaced television cameras with 512 scanning lines can be connected to the C1900-01 directly, thus eliminating the need for a camera control unit. Image preprocessing functions such as integration, subtraction and image enhancement are not available. These processes may be programmed and commanded by a separate computer.

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● Two image analysis systems are available from Artek that can be used in conjunction with the light microscope. The Artek 880 and 980 image analysers allow a choice of scanning field shape, size and position. The systems can analyse three operations — numerical count, object area and aperture area — and these can be reselected using a mode selector. Both the 880 and 980 have bottom light and dark-field illumination capacities. The bottom light can be used when light is able to penetrate the objects being analysed and the darkfield can be used when the contrast between the object and the background is poor. In addition, the 880 has a top light.

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● The Photonic microscope system from Hamamatsu allows organisms such as motile cells to be viewed with an optical microscope, something that had hitherto been difficult. The system can be used with optical microscopes with differential interface contrast facilities. A television camera is fitted to a conventional optical microscope equipped for differential interference contrast (DIC) and controlled by an image processor incorporating video frame memories. A range of image processing functions can be performed including background subtraction, integration, contrast and edge enhancements.

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● The Zeiss Videoplan 2 is an interactive image analysis system which can perform quantitative analysis of geometric characteristics and data evaluation in the life and material sciences. The system evaluates photographic materials, including X rays, and is equally effective in analysing direct images from video images or light and electron microscopes. Videoplan 2 consists of a digitizer tablet, a microcomputer, a video monitor and a portable keyboard. Dual, double-sided, high-density disk drives and a 64-K user memory plus a 16-K video memory are included. As a stand-alone system, Videoplan 2 can be programmed for turn-key use or it can function as a data generator that interfaces with other computers. The updated Videoplan 2 software includes an array of data acquisition programs, statistics, stereology, data editing and graphic routines for distribution and correlation analyses. Specialized programs are available as options. The computer uses a CP/M operating system and can be programmed in several languages.

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The Videoplan 2 interactive image analysis system from Zeiss

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.



Pye Unicam's colour measurement system

● A new colour measurement system is available from Pye Unicam. It is based on the PU 8800 spectrophotometer which uses a software package for the HP 85 computer. The PU 8800 range of instruments incorporates a video display, a built-in recorder and printer options and a choice of side or end-window photo-multipliers. The colour measurement software is configured for operation with an HP 85 and coupled through the PU 8800's bi-directional RS 232C interface. Available on tape and disk, the software provides measurement of colour or colour difference in up to three modes — diffuse or total reflectance and transmission. Illuminants A, C, D₆₅ and D₇₅ are offered, all weighted at 2° and 10° observer angles. Reflectance data on up to 99 standards or samples can be stored on file for immediate recall and the system allows editing of stored black and white calibration values if required. The results can be produced in both tabular and graphical form, along with a listing of the system and calculation conditions, operator identity and date. The instrument can store up to ten user programs.

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● Micromeritics has produced an interface for linking a computer to its SediGraph system. The interface, called SediComp, is designed to allow any SediGraph 5000ET to be adapted to Apple II+ and Apple IIe microcomputers. SediComp expands the SediGraph data output capability so that it can provide tabular and graphic data in printed or video display format. Programs and data are stored on standard 5¼-inch floppy disks. The SediGraph automates the classical sedimentation technique. The unit can analyse particle sizes in the range from 0.1 µm to 100 µm in a single analysis. The SediComp package includes Apple personal computer boards, master program disks, connecting cables and a user manual.

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● Zenith Data Systems has launched two monochrome video monitors known as the ZVM-122 and ZVM-123 green phosphor display, depending upon visual preference. The new monitors are compatible with most business microcomputers and video games, such as the Apple II and III, the IBM personal computer, the Commodore 64 and Vic 20, Texas Instruments' 99/4 and the Atari 800 and 1200, as well as Zenith's own Z100 desktop computer. Designed with a bandwidth of 15 MHz that gives the ability to display more than 800 lines horizontally, the ZVM-122/123 monitors have a rise time of 30 ns. The instrument's 12-inch diagonal screen offers a display with up to 25 lines containing 80 characters each. Each monitor is also equipped with a 40 to 80 character switch. This enables the character size to be increased to accommodate a video format when the computer has a 40-column display.

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● The Hamamatsu C1901 and C1901-01 are 16-bit video frame memories with real-time image preprocessing functions. The C1901 is intended for use with raster-scan television cameras, including Hamamatsu's C1000 series and the C1145 range of non-interlaced TV cameras with 512 scanning lines. Since the C1901 contains its own synchronous generator, it is directly compatible with the camera head of the C1000 or C1145. A camera control unit is not required. In contrast, the C1901-01 is designed for use with Hamamatsu's C1181-01 random access camera (image dissector camera) operating in the photon counting mode. It can also be modified for slow scan inputs for the scanning electron microscope. Both models have real-time image preprocessing functions such as integration subtraction, shading correction and image enhancement controlled by using keyboard strokes. In addition, programmable red, green and blue (RGB) outputs are provided.

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● Micro Consultants has introduced IDAS, a new generation of digital fluorography equipment. IDAS is a digital fluorography system that interfaces directly to any modern X-ray equipment. The fluoroscopic television output is connected to IDAS via a single coaxial cable. The system is mobile, being trolley mounted. It uses the graphics technology of Micro Consultants' sister company Quantel, and can be expanded with modular software packages. IDAS digital image storage capability can be expanded by interfacing to Centinel, MicroConsultants' medical central image library system. Centinel provides a digital centralized storage and filing system for both pictures and patient records. Images can be stored on Winchester disks and on removable cartridge disks. It can support a number of remote image terminals which have fast access to 500,000 (base system) digitally-stored images.

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IDAS from Micro Consultants

● Dapple System's Imageplus+ is a software-based image analysis system that can be used in conjunction with a standard Apple IIe computer with 64 K of RAM. The system is boosted with hardware and software options that provide a 256 K memory so that the computer operates at over three times the speed of a standard Apple IIe! This increased capability enables all 64 grey levels to be acquired and stored simultaneously. The system first acquires the image through a video camera, then provides measurements such as area, length, breadth, perimeter and orientation of each feature. Facilities are provided for both manual and automatic modification of the raw image data. Imageplus+ can also provide a range of statistical and graphical operations. These include correlation between variables, means, variances and multiple regression. Imageplus+ is available in Europe from Miller Systems.

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